

CHAPTER IV

Results

Part I Study on estrogenic activity in plants.

Table 1 (A,B) demonstrates dose response curves of standard estradiol (E_2) and standard estrone (E_1)(figure 16). Various doses of standard estrogens were subcutaneously injected to immature female mice in divided dose for 3 days successively.

Estradiol : Five dose levels were employed, ranging from 0.01 to 0.16 μg , steep part of dose response curve lies between 0.015 to 0.06 μg .

Estrone : Five dose levels were also employed, ranging from 0.02 to 0.32 μg , steep part of dose response curve lies between 0.04 to 0.16 μg .

Estriol : Five dose levels were employed, ranging from 0.01 to 0.16 μg , steep part of dose response curve was not shown.
(figure 16 a)

Table 1 A,B

	Control	dose (μg)				
		0.01	0.02	0.04	0.08	0.16
<u>Std.Estradiol (E₂)</u>						
Uterine wt.(mg) mean \pm SD	20.1 \pm 0.7	20.6 \pm 4.4	32.3 \pm 8.8	48.8 \pm 4.1	61.7 \pm 5.7	67.3 \pm 3.6
No. of mice	10	9	9	8	7	8
<u>Std.Estriol (E₃)</u>						
Uterine wt.(mg) mean \pm SD	9.9 \pm 2.8	10.2 \pm 2.5	15.0 \pm 4.1	12.4 \pm 1.5	14.5 \pm 2.8	14.2 \pm 3.5
No. of mice	12	11	10	8	10	10

	Control	dose (μg)				
		0.02	0.04	0.08	0.16	0.32
<u>Standard Estrone</u> (E ₁)						
Uterine wt.(mg) mean \pm SD	10.8 \pm 1.1	15.2 \pm 1.3	19.5 \pm 1.7	27.7 \pm 1.2	37.2 \pm 1.6	41.6 \pm 3.2
No. of mice	5	5	4	4	5	5

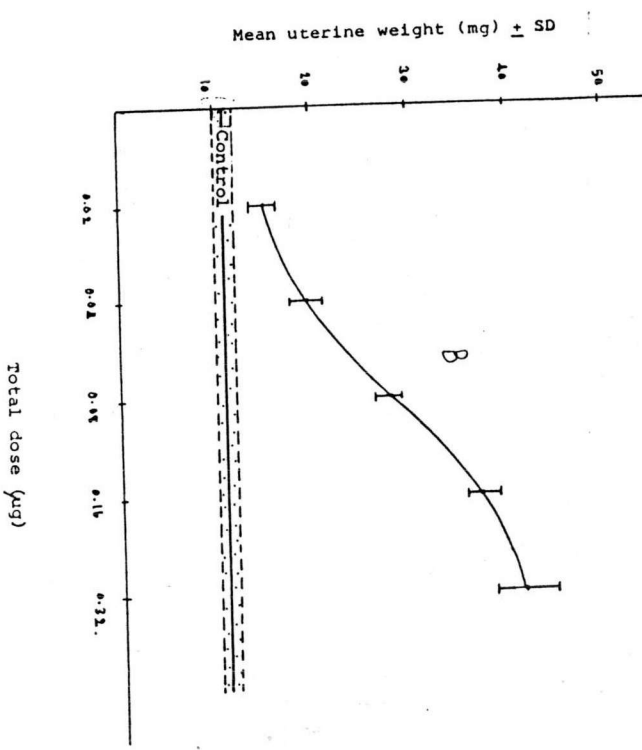
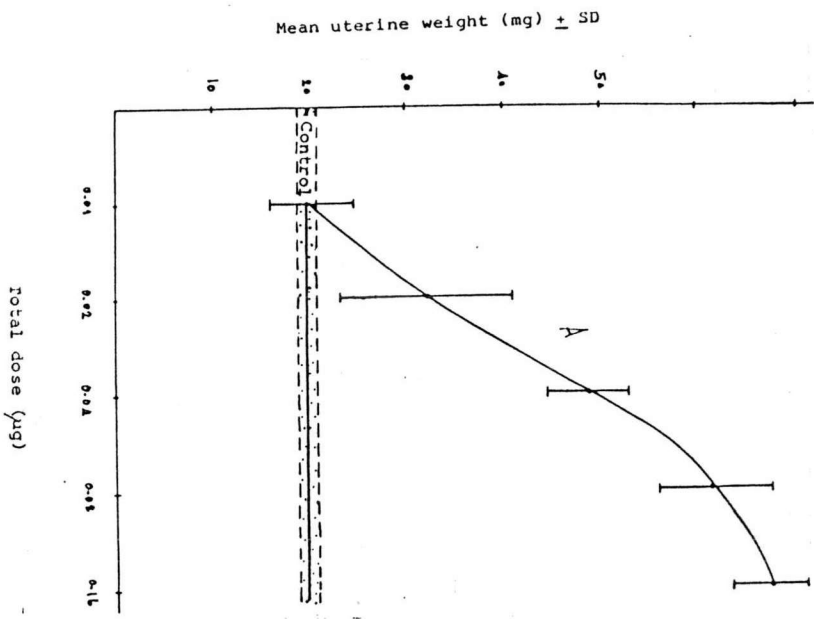


Figure 16 Demonstrate a long range standard curve for standard estrogen
 A. Estradiol (E₂).
 B. Estrone (E₁).

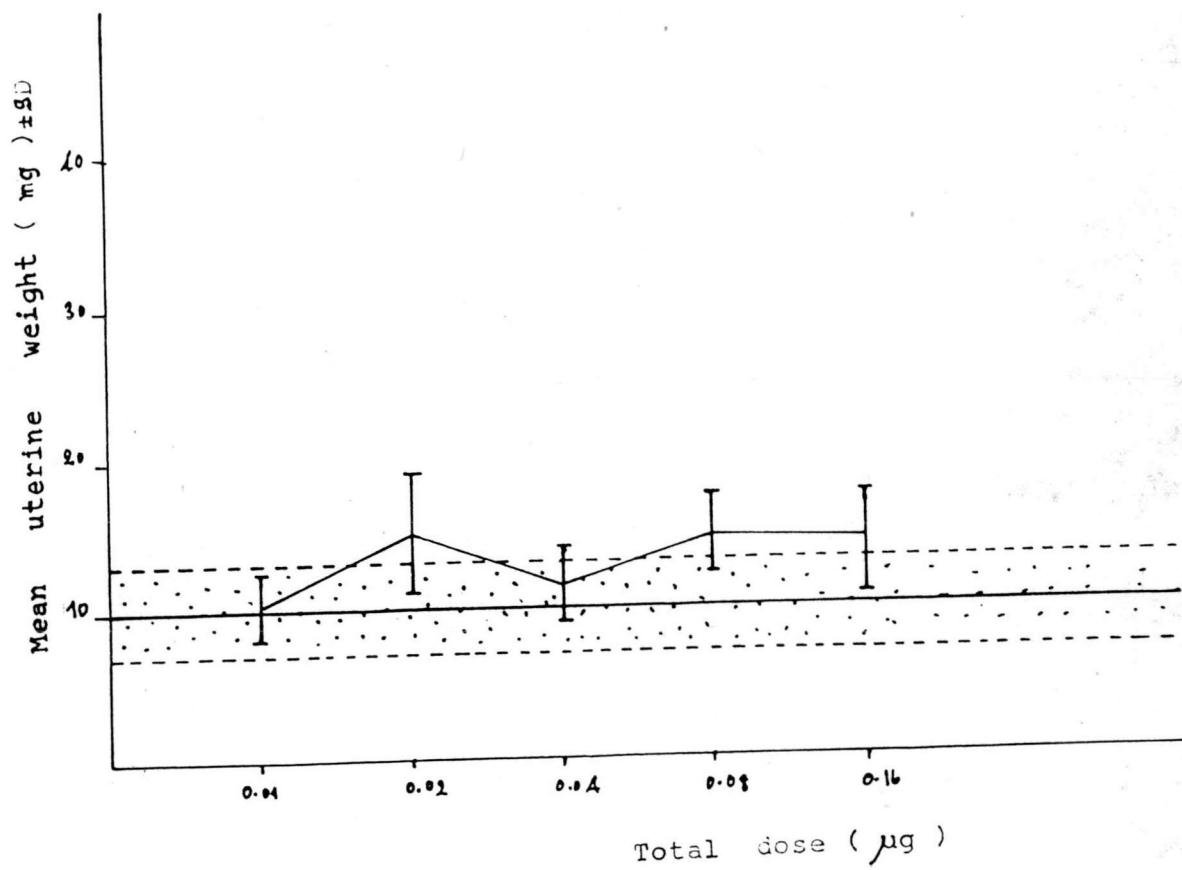


Figure 16a Demonstrate a long range standard curve of estriol (E₃)

Table 2 Demonstrates the results obtained from variety of plants tested, in which increase in weight, occurrence of vagina opening and cornification of vaginal epithelium were observed :

There were no alteration in uterine weight and other parameters in Ocimum basilicum, Ocimum sanctum, Alpinia galanga, Zingiber cassumunar, Citrus hystrix, Gastrochilus panduratus, Momordica charantia both large and small variety (see figure 17, 18, 20, 21 p. 75, 76, 78, 79, 81).

Volatile oil of Cympobogon citratus did not show a uterotropic activity, alcohol extract induced uterine enlargement in the first experiment. When the experiment was repeated, no activity was observed (see figure 19 & 23 p. 77, 81). Piper betel and Vigna sesquipedalis induced uterine enlargement in the first experiment when the experiment was repeated, there was little activity as calculated in term of standard estrone (see figure 19& 22 p. 77 & 80).

Piper betel 1.04 mg extract which derived from 2.4 gm of fresh plants, relative potency when expressed as standard estrone was 0.0257 μ g. Repeat of this experiment, 0.52 mg of extract which is equivalent to 1.2 gm of fresh plant possess estrogenic activity equal to 0.0239 μ g of standard estrone.

Alcoholic extract of Vigna sesquipedalis possessed estrogenic activity. Relative potency expressed as standard estradiol was 0.0202 μ g from 2.15 mg of extract which equivalent to

0.088 gm of fresh plant. Repeat this experiment using standard estrone, relative potency was 0.0335 μg estrone in 0.54 mg of extract which equivalent to 0.022 gm of fresh plant. Alcoholic extract of Cymbopogon citratus, at low dose, possessed estrogenic activity, and relative potency expressed as standard estradiol was 0.0259 μg in 3.99 mg of extract which is equivalent to 0.11 gm fresh plant.

Table 2.

Plants extracts	Parameter	Control	Std. E ₂ doses (µg)		Std. E ₁ doses (µg)		Unknown doses (mg)			Potency of unknown	Design of assay	Index of precision
			0.02	0.06	0.04	0.12	0.5	1.5	4.5			
<i>O. basilicum</i> (oil) Fig. 17 p. 75	No. of mice	6	7	6	5	5	6	6	6	none	-	-
	Uterine wt. (mg) mean ± SD	12.2±4.6	21.9±4.3	39.0±4.3	18.6±2.5	27.8±3.8	16.4±2.8	13.2±3.9	11.4±2.6			
	Vag. opening	-	+	+	+	+	-	-	-			
	Vag. cornification	-	+	+	+	+	-	-	-			
Plants extracts	Parameter	Control	Std. E ₂ doses (µg)		Std. E ₁ doses (µg)		Unknown doses (mg)		Potency of unknown	Design of assay	Index of precision	
			0.015	0.06	0.02	0.08	0.08	0.3				
<i>O. sanctum</i> (oil) Fig. 18 p. 76	No. of mice	6	7	5	6	5	5	5	none	-	-	
	Uterine wt. (mg) mean ± SD	12.4±2.2	21.2±3.0	36.1±4.6	14.0±5.4	20.8±1.8	9.8±3.7	7.8±1.0				
	Vag. opening	-	-	+	-	+	-	-				
	Vag. cornification	-	-	+	-	+	-	-				
Plants extracts	Parameter	Control	Std. E ₂ doses (µg)		Std. E ₁ doses (µg)		Unknown doses (mg)		Potency of unknown	Design of assay	Index of precision	
			0.015	0.06	0.02	0.08	0.96	3.8				
<i>Zingiber cassumunar</i> Fig 20 p. 78	No. of mice	6	5	7	5	7	7	7	none	-	-	
	Uterine wt. (mg) mean ± SD	12.5±2.6	20.6±2.3	46.2±7.1	13.6±4.8	24.5±5.2	12.3±2.3	11.2±4.3				
	Vag. opening	-	-	+	-	+	-	-				
	Vag. cornification	-	-	+	-	+	-	-				

Table 2. (Cont.)

Plants extracts	Parameter	Control	Std. E ₂ doses (µg)		Std. E ₁ (µg)		Unknown doses (mg)		Potency of unknown	Design of assay	Index of precision
			0.015	0.06	0.02	0.08	2.4	9.6			
<i>Alpinia galanga</i> Fig. 20 p. 78	No. of mice	6	5	7	5	7	7	7	none	-	-
	Uterine wt.(mg) mean ± SD	12.5±2.6	20.6±2.3	46.2±7.1	13.6±4.8	24.5±5.2	14.3±4.6	12.7±1.5			
	Vag. opening	-	-	+	-	+	-	-			
	Vag.cornification	-	-	+	-	+	-	-			
Plants extracts	Parameter	Control	Std. E ₂ doses (µg)		Std. E ₁ (µg)		Unknown doses (mg)		Potency of unknown	Design of assay	Index of precision
			0.015	0.06	0.02	0.08	2.0	8.0			
<i>M. charantia</i> Fig. 20 p. 78	No. of mice	6	5	7	5	7	7	7	none	-	-
	Uterine wt.(mg) mean ± SD	12.5±2.6	20.6±2.3	46.2±7.1	13.6±4.8	24.5±5.2	13.9±4.8	14.3±8.3			
	Vag. opening	-	-	+	-	+	-	-			
	Vag.cornification	-	-	+	-	+	-	-			
Plants extracts	Parameter	Control	Std. E ₂ doses (µg)		Std. E ₁ (µg)		Unknown doses (mg)		Potency of unknown	Design of assay	Index of precision
			0.015	0.06	0.02	0.08	5.8	23.2			
<i>Cymbopogon citratus</i> Fig. 23 p. 81	No. of mice	6	6	5	4	4	6	6	none	-	-
	Uterine wt.(mg) mean ± SD	13.0±4.1	28.9±5.3	46.4±8.3	17.4±3.7	28.1±8.2	12.8±3.7	17.1±8			
	Vag. opening	-	-	+	-	+	-	-			
	Vag.cornification	-	-	+	-	+	-	-			

Table 2. (Cont.)

Plants extracts	Parameter	Control	Std. E ₂ dose (µg)		Std. E ₁ dose (µg)		Unknown doses (mg)		Potency of unknown	Design of assay	Index of precision
			0.015	0.06	0.02	0.08	0.52	2.08			
<u>Piper betel</u> Fig. 21 p. 79	No. of mice	6	6	5	4	4	5	5	0.0257µg of E ₁ in 1.04mgExt (2.4gm fresh plant)	4 point	0.49
	Uterine wt.(mg)										
	mean ± SD	13.0±4.1	28.9±5.3	46.4±8.3	17.4±3.7	28.1±8.2	18.5±3.1	25.3±12.0			
	Vag. opening	-	-	+	-	+	-	-			
	Vag.cornification	-	-	+	-	+	-	-			
Plants extracts	Parameter	Control	Std. E ₁ dose (µg)		Unknown doses (mg)		Potency of unknown	Design of assay	Index of precision		
			0.02	0.08	0.13	0.52					
<u>Piper betel</u> (Repeat) Fig. 19 p. 77	No. of mice	5	5	5	5	5	5	0.0239 of E ₁ in 0.52mgExt (1.2gm fresh plant)	3 point	0.12	
	Uterine wt.(mg)										
	mean ± SD	9.5±3.6	15.2±1.3	27.7±1.2	11.8±1.5	14.0±3.2	-				-
	Vag. opening	-	-	+	-	-	-				-
	Vag.cornification	-	-	+	-	-	-				
Plants extracts	Parameter	Control	Std. E ₂ dose (µg)		Std. E ₁ dose (µg)		Unknown doses (mg)		Potency of unknown	Design of assay	Index of precision
			0.015	0.06	0.02	0.08	1.7	6.8			
<u>Citrus hystrix</u> Fig. 21 p. 79	No. of mice	6	4	4	5	5	5	5	None	-	-
	Uterine wt.(mg)										
	mean ± SD	7.9±0.3	18.6±0.9	42.4±1.8	13.8±4.7	20.8±2.0	8.7±1.6	8.8±0.2			
	Vag. opening	-	-	-	-	+	-	-			
	Vag.cornification	-	-	-	-	+	-				

Table 2. (Cont.)

Plants extracts	Parameter	Control	Std. E ₂ doses (µg)		Std. E ₁ doses (µg)		Unknown doses (mg)			Potency of unknown	Design of assay	Index of precision
			0.015	0.06	0.02	0.08	13.3	40.0				
<u>M. charantia</u> (small variety) Fig. 21 p. 79	No. of mice	6	4	4	5	5	6	6	none	-	-	
	Uterine wt. (mg)											
	mean ± SD	7.9±0.3	18.6±0.9	42.4±1.8	13.8±4.7	20.8±2.0	9.6±1.9	9.0±1.2				
	Vag. opening	-	-	+	-	+	-	-				
	Vag. cornification	-	-	+	-	+	-	-				
Plants extracts	Parameter	Control	Std. E ₂ doses (µg)		Unknown doses (mg)			Potency of unknown	Design of assay	Index of precision		
			0.02	0.08	0.8	3.2	12.9					
<u>Gastrochilus panduratus</u> Fig. 23 p. 81	No. of mice	5	5	6	6	6	6	none	-	-		
	Uterine wt. (mg)											
	mean ± SD	16.0±2.9	29.3±4.5	32.9±8.6	15.4±4.3	18.1±5.4	14.2±3.8					
	Vag. opening	-	-	+	-	-	-					
	Vag. cornification	-	-	+	-	-	-					
Plants extracts	Parameter	Control	Std. E ₂ doses (µg)		Unknown doses (mg)			Potency of unknown	Design of assay	Index of precision		
			0.02	0.08	4.0	16.0	64.0					
<u>Cymbopogon citratus</u> (Alcohol extract) Fig. 23 p. 81	No. of mice	5	5	6	6	6	6	0.0259 µg of E ₂ in 3.99mg Ext (0.11gm fresh plant)	3 point	0.37		
	Uterine wt. (mg)											
	mean ± SD	16.0±2.9	29.3±4.5	32.9±8.6	23.3±10.8	11.3±2.8	11.6±0.6					
	Vag. opening	-	-	+	-	-	-					
	Vag. cornification	-	-	+	-	-	-					

Table 2. (Cont.)

Plants extracts	Parameter	Control	Std. E ₁ doses (µg)		Unknown doses (mg)		Potency of unknown	Design of assay	Index of precision		
			0.04	0.16	1.0	4.0					
<i>Cymbopogon citratus</i> (Repeat) Fig. 23 p. 81	No. of mice	5	4	5	5	5	none	-	-		
	Uterine wt.(mg) mean ± SD	10.8±1.1	19.5±1.7	37.2±1.6	12.4±1.7	10.0±1.2					
	Vag. opening	-	+	+	-	-					
	Vag.cornification	-	+	+	-	-					
Plants extracts	Parameter	Control	Std. E ₂ doses (µg)		Unknown doses (mg)				Potency of unknown	Design of assay	Index of precision
			0.015	0.06	0.54	2.15	8.58	34.0			
<i>Vigna sesquipedalis</i> Fig. 22 p. 80	No. of mice	5	5	6	5	5	5	6	0.0202 µg of E ₂ in 2.15 mgExt (0.09 gm fresh plant)	3 point	0.18
	Uterine wt.(mg) mean ±SD	19.3±2.8	25.2±2.7	57.1±8.8	20.1±3.7	28.5±7.5	20.3±5.6	23.2±7.4			
	Vag. o	-	-	+	-	-	-	-			
	Vag.cornification	-	-	+	-	-	-	-			
Plants extracts	Parameter	Control	Std. E ₁ doses (µg)		Unknown doses (mg)		Potency of unknown	Design of assay	Index of precision		
			0.04	0.16	0.54	2.15					
<i>Vigna sesquipedalis</i> (Repeat) Fig. 22 p. 80	No. of mice	5	4	6	5	5	0.0335 µg of E ₁ in 0.54 mgExt (0.02gm fresh plant)	3 point	0.09		
	Uterine wt.(mg) mean ± SD	10.8±1.1	19.5±1.7	37.2±1.6	14.0±9.0	11.6±3.0					
	Vag. opening	-5	+	+	-	-					
	Vag.cornification	-	+	+	-	-					

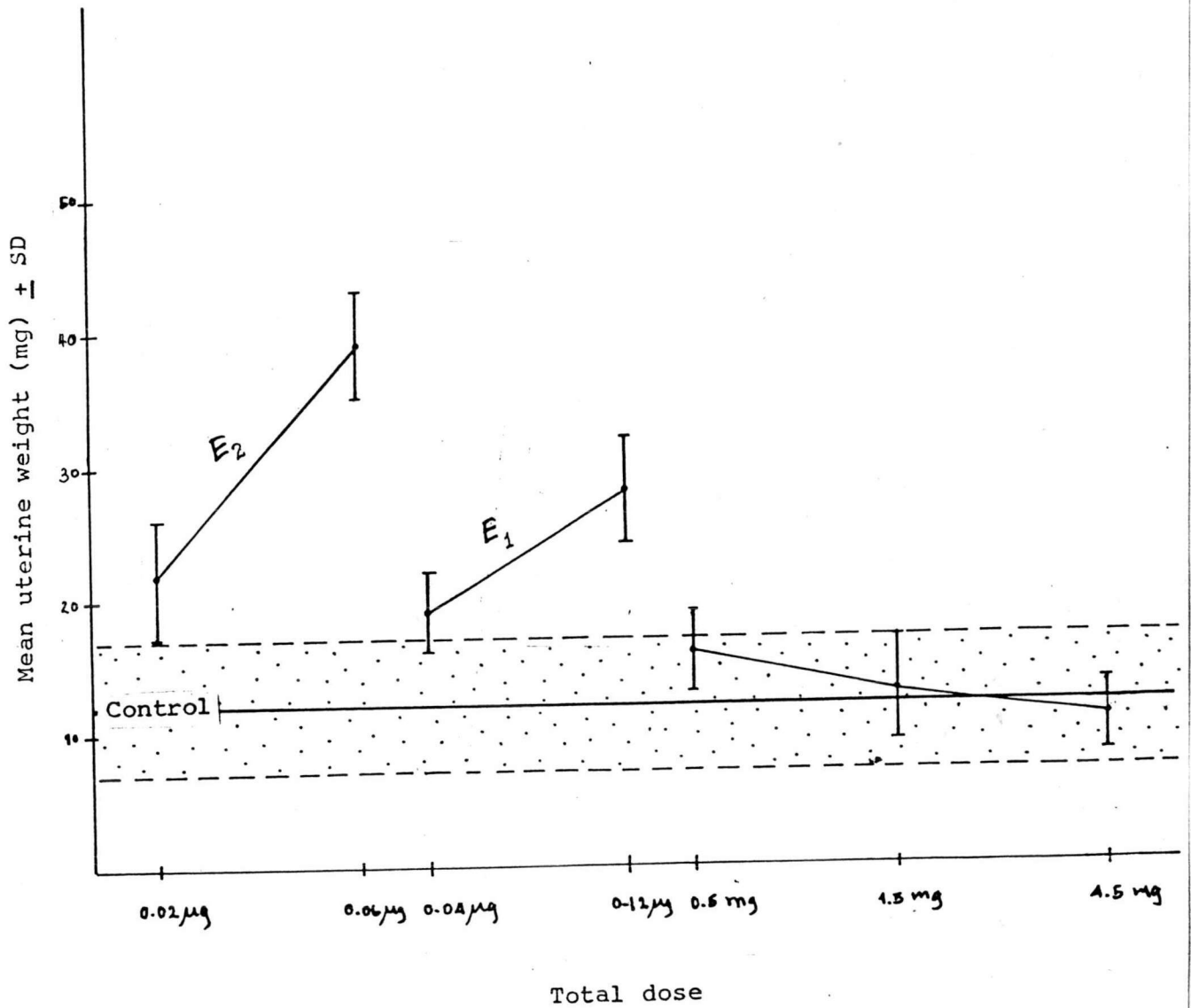


Figure 17 Demonstrates the results obtained from estrogenic activity testing of Ocimum basilicum.

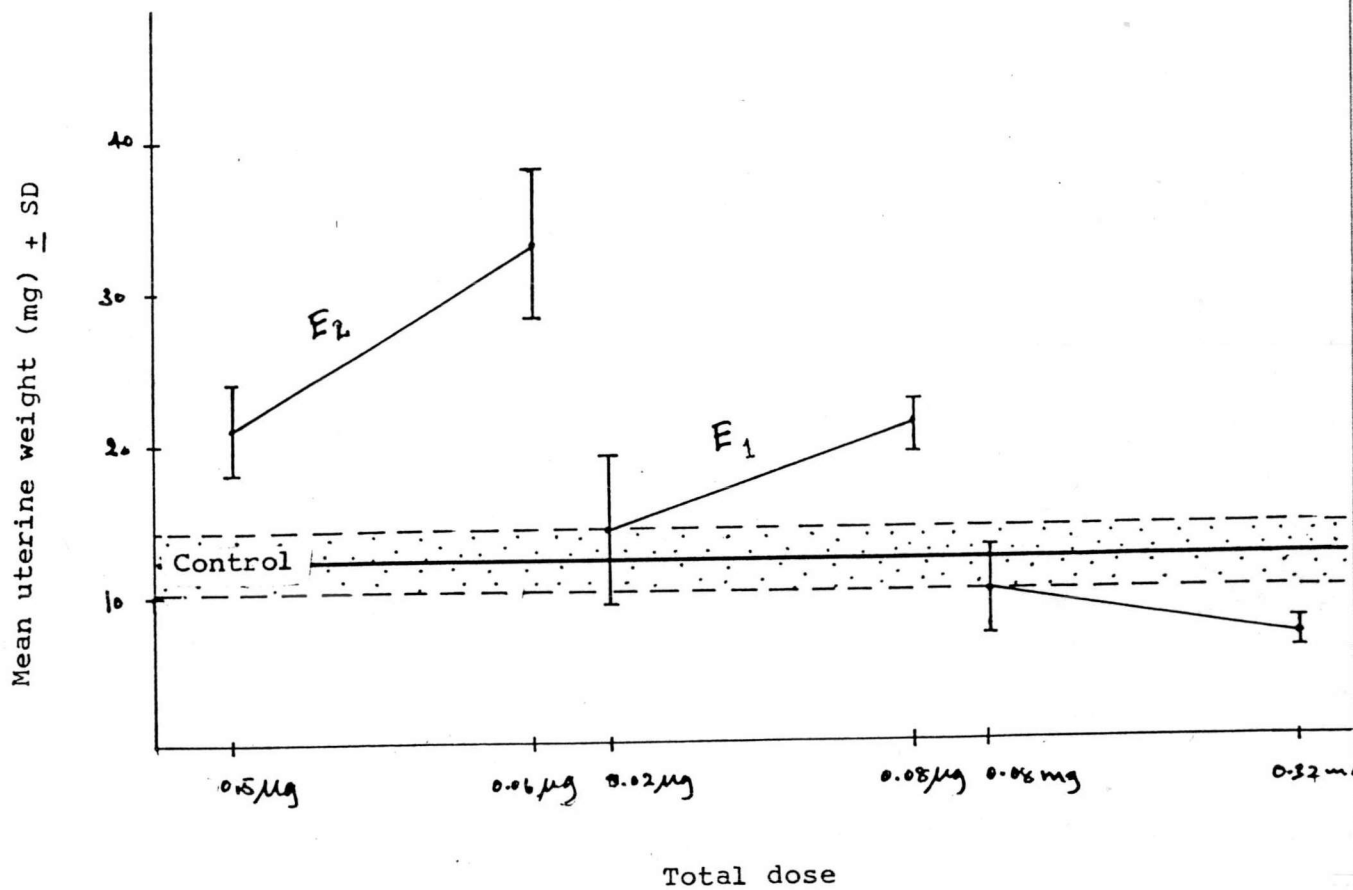


Figure 18 Demonstrates the results obtained from estrogenic activity testing of Ocimum sanctum.

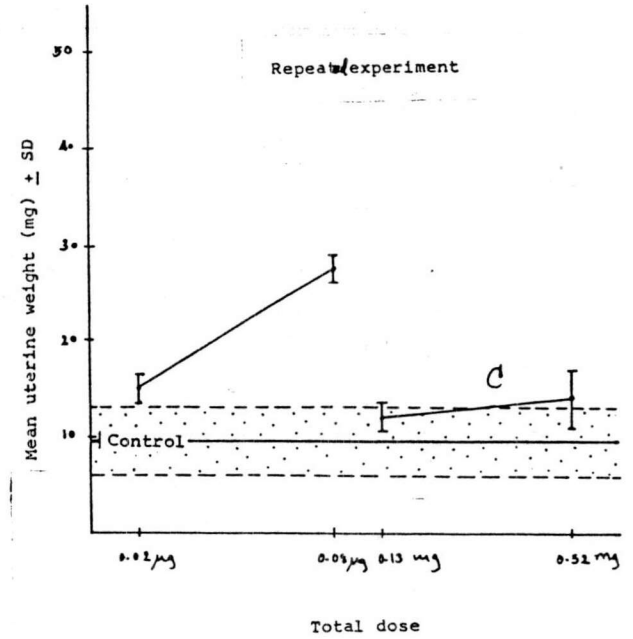
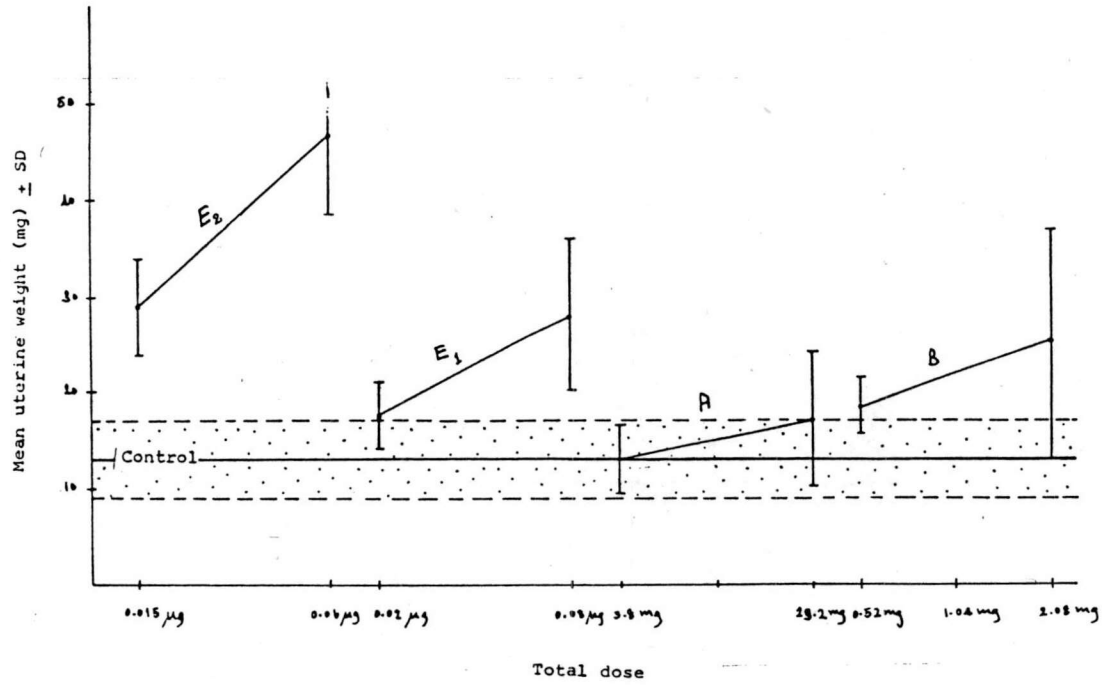


Figure 19 Demonstrate the results obtained from estrogenic activity testing of :
 A. Cymbopogon citratus (oil). B. Piper betel.
 C. Piper betel repeated.

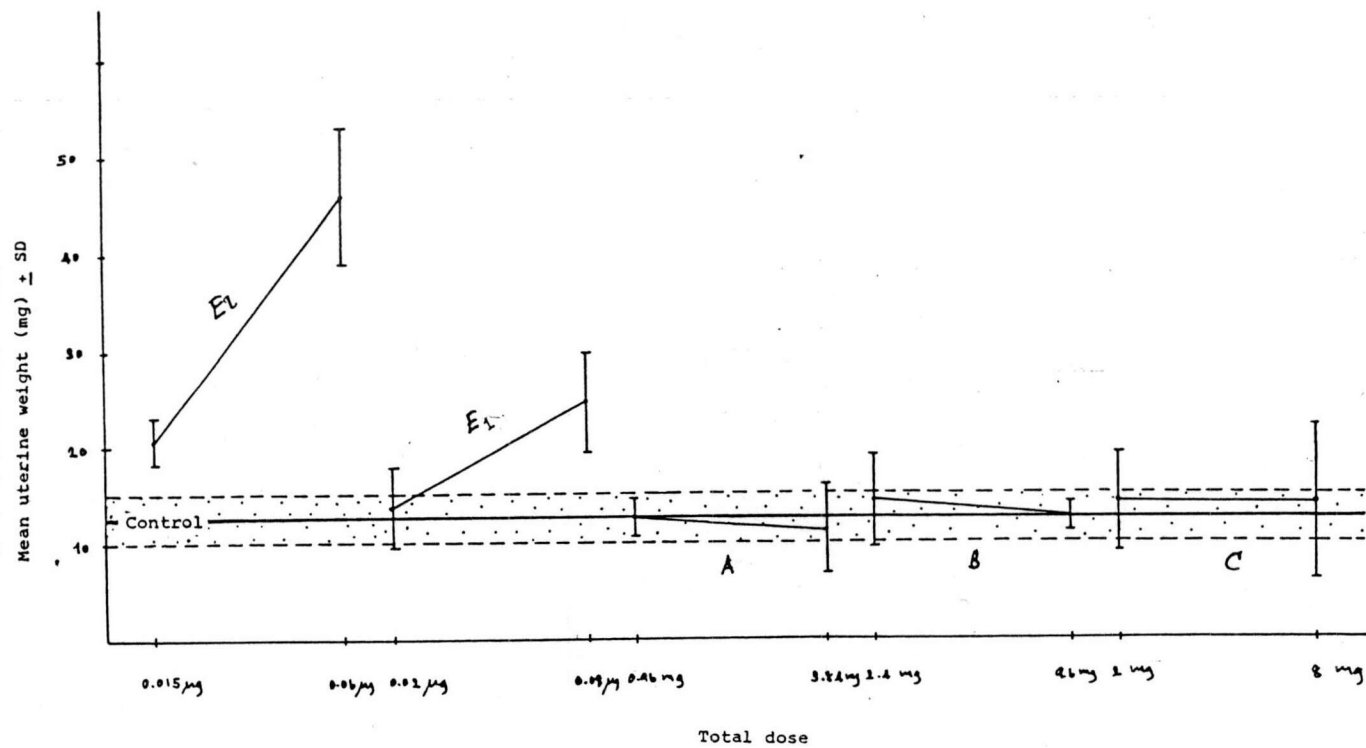


Figure 20 Demonstrate the results obtained from estrogenic activity testing of :

A. Zingiber cassumunar.

B. Alpinia galanga.

C. Homerdica charantia (large variety).

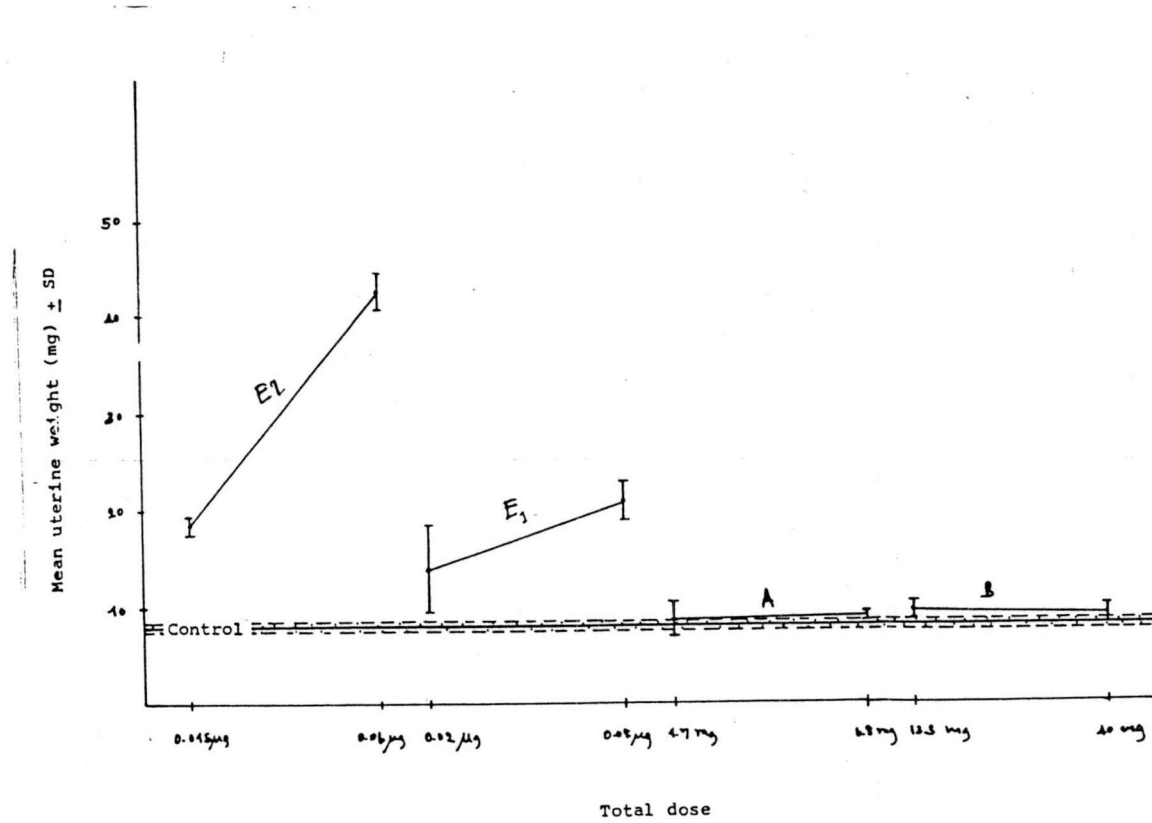


Figure 21 Demonstrate the result obtained from estrogenic activity testing of
 A. Citrus hystrix. B. Momordica charantia (small variety).

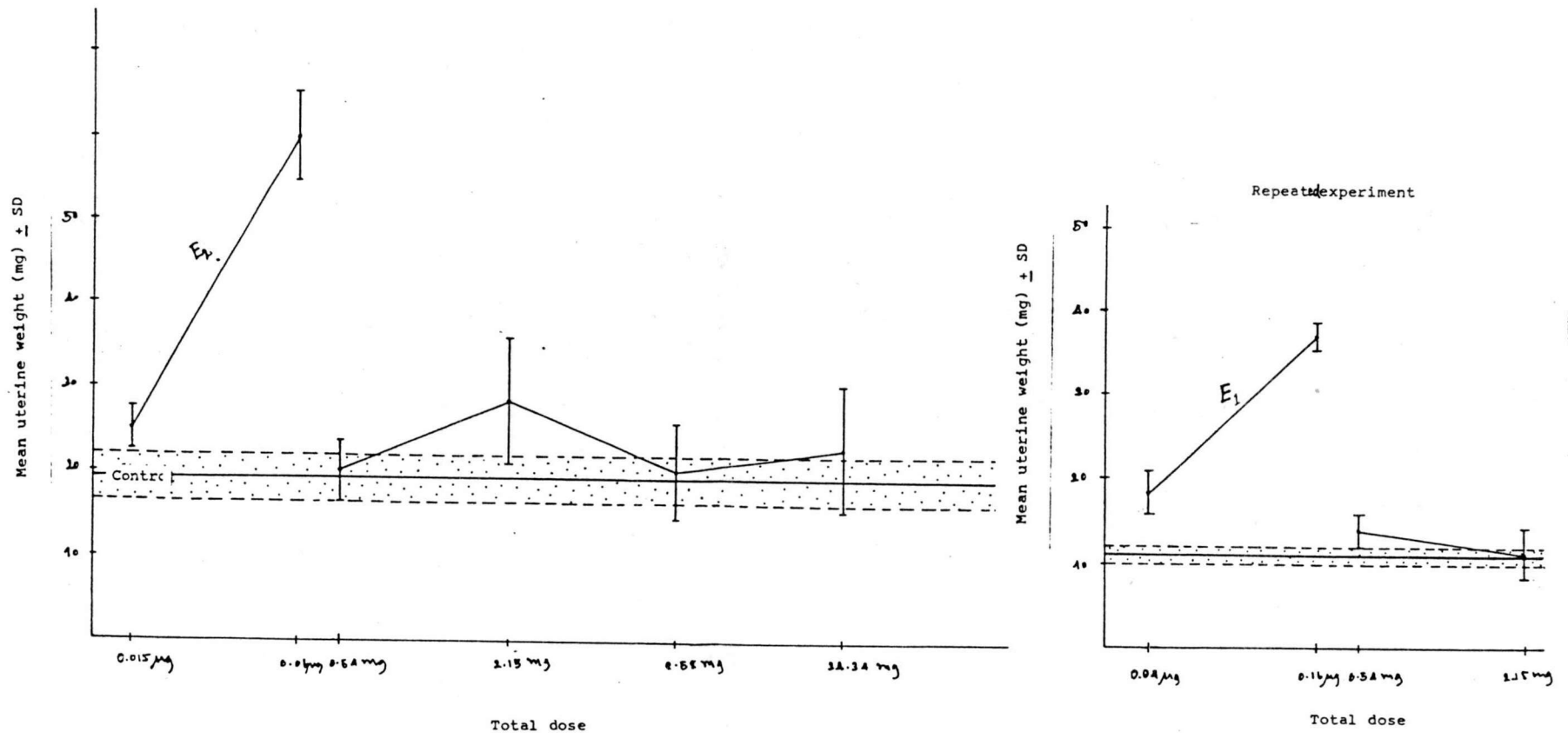


Figure 22 Demonstrates the results obtained from estrogenic activity testing of Vigna sesquipedalis.

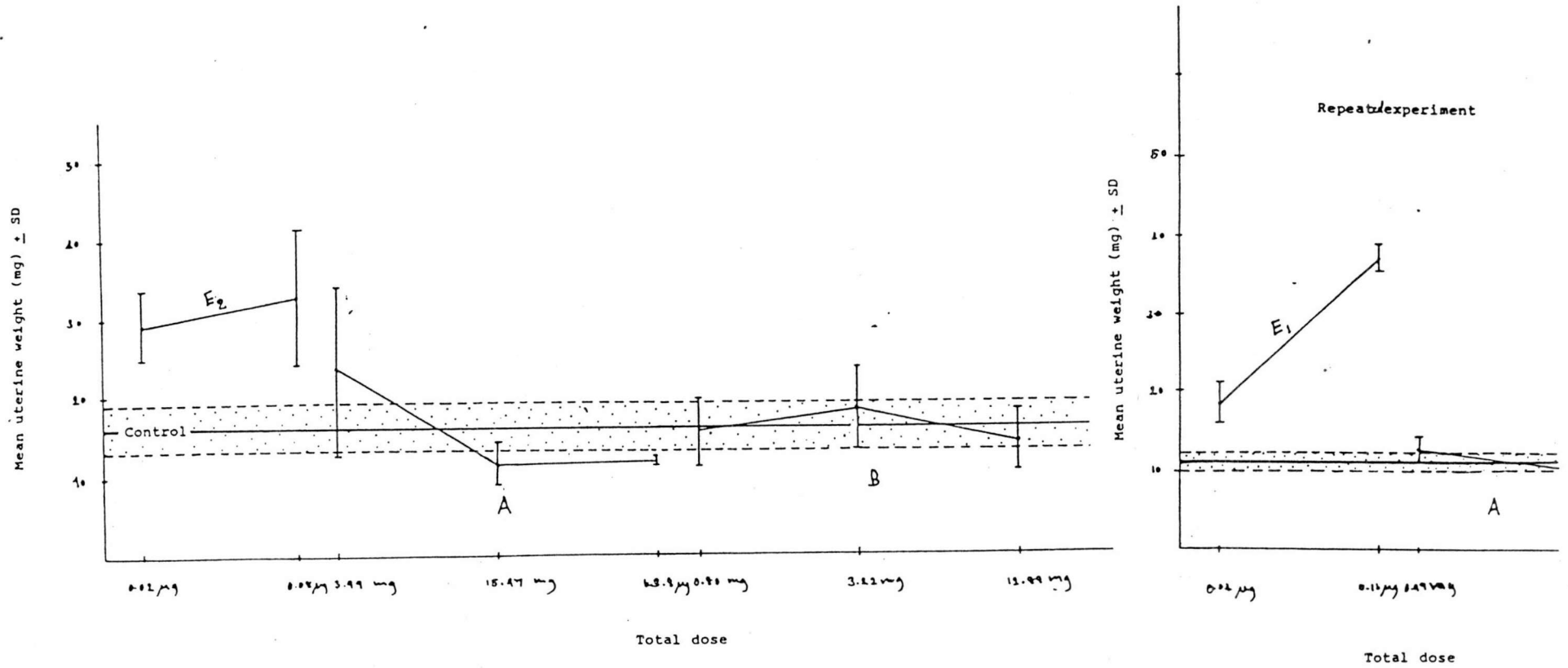


Figure 23 Demonstrates the results obtained from estrogenic activity testing of
 A. *Cymbopogon citratus* (alcohol extract).
 B. *Gastrochilus panduratus* (alcohol extract).

Part II Study on antispermatogenic effect of various plant extracts
in rats and mice.

Table 3-6 showed the results obtained from antispermatogenic study of M. charantia (small variety).

Table 7-10 showed the results obtained from antispermatogenic study of M. charantia (large variety).

Table 11-18 showed the results obtained from antispermatogenic study of O. basilicum.

Table 19-22 showed the results obtained from antispermatogenic study of O. sanctum.



Experiment 1 Effects of alcoholic extract from the fruit of M. charantia (small variety) on spermatogenesis in mice.

Table 3 Demonstrates the results obtained from expt. 1 in which the extract was given orally*daily in dosage of 400 mg/kg/day for a period of 15 days. Total sperm count, sperm motility, weight of testis, epididymis, prostate&seminal vesicles in control and experiment groups were shown. There was no statistical significant difference in all of the parameters measured. 37.5%, 30% of mating positive was observed in control and experiment groups respectively.

Table 4

No. of mice	Mating	Total sperm count (mill)	Sperm motility			Weight (mg)			Body wt. (gm)	
			%	grade	epid.	testes	SV & total prost.	start	end	
<u>Control</u>										
1	-	27.3	63	3	32.0	105.0	262.6	27.5	29.3	
2	-	21.2	50	2	34.0	82.0	336.0	30.0	32.5	
3	-	20.2	35	1	28.0	94.0	274.0	29.5	33.5	
4	-	24.7	34	1	40.0	114.0	360.0	30.7	35.0	
5	+	19.4	40	1	42.8	110.0	390.8	28.5	34.0	
6	+	27.2	49	3	37.6	120.0	228.6	29.8	29.5	
7	-	21.0	55	1	36.0	112.0	251.0	28.7	27.0	
8	-	22.6	58	2	39.8	112.8	336.4	28.6	37.0	
9	-	18.7	14	1	40.0	114.5	419.0	28.3	23.0	
22.2%										
Mean		22.5	44.2	1.7	36.7	107.1	320.9	29.1	32.3	
SEM		1.1	5.0	0.3	1.6	3.9	22.8	0.3	1.0	
<u>Experiment</u>										
1	-	20.1	40	2	32.0	103.2	348.4	29.5	31.0	
2	-	15.3	57	3	33.0	102.2	280.0	28.5	33.5	
3	-	19.1	47	2	39.6	125.0	326.6	31.6	34.0	
4	-	18.8	39	1	38.0	114.2	422.4	30.5	34.0	
5	-	12.8	36	1	28.0	92.0	151.0	30.0	29.0	
6	-	19.7	35	1	34.0	103.8	256.6	27.5	28.0	
7	-	17.6	41	2	34.0	98.4	352.8	28.0	32.0	
8	-	19.2	45	3	31.0	104.0	306.0	27.5	28.5	
0%										
Mean		17.8	42.5	1.9	33.7	105.3	305.5	29.1	31.2	
SEM		0.9	2.5	0.3	1.3	3.6	28.4	0.5	0.9	
t-test		$p < 0.01$	NS	NS	NS	NS	NS	NS	NS	

Table 4

Demonstrates the results obtained from experiment 1, extract was given orally and daily in dose of 400 mg/kg/day for a period of 60 days. There was a statistical significant reduction in total sperm count ($p < 0.01$), while other parameters were not altered. Only control group showed a 22.2% of mating positive.

Table 5

Demonstrates the results obtained from expt. 1 the extract was given orally and daily in dose of 800 mg/kg/day for a period of 15 days. There was no statistical significant difference in all the parameters measured. Moreover, percentage of mating positive animals was not reduced in experimental group.

Table 6

Demonstrates the results obtained from expt. 1 the extract was given orally and daily in dose of 800 mg/kg/day for a period of 60 days. There was no statistical significant difference in all of the parameters measured. 88.9%, 80% of mating positive was observed in control and experiment groups respectively.

Experiment 2 Effects of alcoholic extract from the fruit of M. charantia (large variety) on spermatogenesis in mice.

Table 7 Demonstrates the results obtained from expt. 2. The extract was given orally and daily in dose of 200 mg/kg/day for a period of 15 days. There was no statistical significant difference in all of the parameters measured. Percentage of mating positive animals was not reduced in experimental group.

Table 8

Demonstrates the results obtained from expt.

The extract was given orally and daily in dose of 200 mg/kg/day for a period of 60 days. There was no statistical significant difference in all of the parameters measured. 30%, 10% of mating positive was observed in control and experiment groups respectively.

Table 9

No. of mice	Mating	Total sperm count (mill)	Sperm motility			Weight (mg)		Body wt. (gm)	
			%	grade	epid.	testes	SV & total prost.	start	end
<u>Control</u>									
1	-	20.9	35	2	26.2	91.0	400	27.5	30.4
2	-	30.2	46	3	32.6	130.0	335	30.0	33.0
3	+	20.5	40	2	28.4	97.6	350	29.5	32.0
4	-	27.4	41	2	30.0	106.4	354	30.7	33.0
5	-	25.5	42	2	30.0	110.0	346	28.5	31.7
6	+	25.5	50	2	29.0	116.0	385	29.8	31.7
7	-	14.4	47	2	28.0	100.0	288	28.7	34.2
8	-	22.5	52	3	26.0	88.0	300	27.6	30.0
9	-	16.4	55	3	27.0	66.0	300	28.3	30.8
10	+	18.9	46	2	28.0	103.0	352	28.7	30.5
30%									
Mean		22.2	45.4	2.3	28.5	100.8	341	28.9	31.7
SEM		1.6	1.9	0.1	0.6	5.5	11.6	0.3	0.4
<u>Experiment</u>									
1	+	17.5	42	2	30.0	99.0	360.0	29.5	30.0
2	-	26.0	51	3	25.0	90.0	205.8	28.5	24.5
3	-	26.1	49	2	34.0	112.0	266.4	31.6	32.5
4	+	18.5	50	2,3	30.0	116.2	218.0	30.5	34.0
5	-	20.5	43	2	28.0	112.0	224.0	30.0	33.6
6	-	22.4	56	3	30.0	110.0	302.0	27.5	30.2
7	+	27.5	48	3	33.0	102.0	330.0	28.0	31.8
8	-	15.4	65	3	32.4	106.0	295.0	27.5	30.8
9	-	22.5	42	2	31.0	105.0	330.0	29.5	30.5
10	-	16.0	52	2	33.6	102.0	286.0	27.0	30.5
30%									
Mean		21.2	49.8	2.4	30.7	105.4	281.7	28.9	30.8
SEM		1.4	2.2	0.2	0.9	2.4	16.6	0.5	0.8
t-test		NS	NS	NS	NS	NS	$S_p < 0.01$	NS	NS

Table 9

Demonstrates the results obtained from expt. 2. The extract was given orally and daily indose of 400 mg/kg/day for a period of 15 days. There was a statistical significant reduction in weight of seminal vesicle and prostate ($p < 0.01$) but other parameters measured were not altered. Percentage of mating positive animals was not reduced in experiment group.

Table 10

No. of mice	Mating	Total sperm count (mill)	Sperm motility		Weight (mg)			Body wt. (gm)	
			%	grade	epid.	testes	SV & total prost.	start	end
<u>Control</u>									
1	-	12.7	26	1	38	142	422	38.0	39.2
2	+	20.5	35	1,2	42	116	327	34.0	40.0
3	+	18.0	35	1	40	98	322	31.8	40.8
4	-	19.6	40	1	39	97	384	39.5	35.0
5	-	24.4	43	2	43	107	494	37.8	38.7
6	-	18.7	47	2	41	120	387	36.5	37.0
7	-	15.6	45	3	32	110	274	29.5	31.5
8	-	15.6	31	1	34	100	362	31.8	32.5
25%									
Mean		18.1	37.7	1.6	38.6	111.2	371.5	34.9	36.8
SEM		1.3	2.6	0.3	1.4	5.3	23.9	1.3	1.2
<u>Experiment</u>									
1	-	20.8	35	2	37	100	242	32.3	35.8
2	-	17.9	30	2	38	118	276	35.0	36.2
3	-	17.5	40	2	32	90	281	33.5	35.0
4	-	23.4	39	2	40	116	333	31.8	38.3
5	+	16.5	52	3	42	116	328	35.6	36.2
6	-	16.0	42	2	40	106	337	36.4	37.5
7	-	17.0	42	2	36	108	243	35.4	36.7
8	-	26.4	41	2	40	122	238	32.7	35.0
12.5%									
Mean		19.4	40.1	2.1	38.1	109.5	284.7	34.1	36.3
SEM		1.3	2.2	0.3	1.1	3.8	15.1	0.6	0.4
t-test		NS	NS	NS	NS	NS	$S_p < 0.01$	NS	NS

Table 10

Demonstrates the results obtained from expt. 2.

The extract was given orally and daily in dose of 400 mg/kg/day for a period of 60 days.

Weight of seminal vesicle and prostate was shown to be statistical significantly reduced ($p < 0.01$) while other parameters were not changed. 25%, 12.5% of mating positive was observed in control and experiment groups respectively.

Experiment 3 Effects of volatile oil of sweet basil on spermatogenesis in rats.

Table 11 Demonstrates the results obtained from expt. 3 showing total sperm count, sperm motility, weight of testis, cauda epididymis, seminal vesicle and prostate and ventral prostate in control & experiment groups. The oil was given orally in dose of 145.6 mg/kg/day (equivalent to 50 gm fresh plant/kg/day) for continuous 15 days. There was no statistical significant difference in all of the parameters measured. 16.7%, 20% of mating positive was observed in control and experiment groups respectively.

Table 12

Demonstrates the results obtained from expt. 3.

The oil was given orally in dosage of 145.6 mg/kg/day (equivalent to 50 gm fresh plant/kg/day) for continuous 60 days. There was no statistical significant difference in all of the parameters measured. 33.3%, 16.7% of mating positive animals was observed in control and experiment groups respectively.

Table 13

No. of rats	Mating	Total sperm count (mill)	Sperm motility % grade	Weight (mg)				Body wt. (gm)		
				cauda epid.	testes	SV& total prost.	VP	start	end	
<u>Control</u>										
1	-	100.5	70	2	88.0	1896.0	2361.0	493.0	335	350
2	+	52.0	76	3	54.0	1627.6	2205.6	426.6	285	295
3	-	132.5	59	2	90.0	1731.4	2406.2	568.8	313	327
4	-	54.0	50	1	83.2	1563.6	2485.6	521.6	290	302
5	+	128.0	65	3	70.0	1565.6	2469.4	485.4	311	323
6	-	75.0	63	3	84.0	1719.2	2301.8	452.6	303	330
33.3%										
Mean		90.3	63.8	2.3	78.2	1683.9	2371.6	491.3	306.2	322
SEM		14.5	3.7	0.3	5.6	51.7	43.3	20.5	7.3	8.8
<u>Experiment</u>										
1	-	56.0	62	1	71.0	1405.0	1699.0	374.0	298	305
2	+	41.0	53	1	43.4	2843.8	1516.8	286.0	305	315
3	-	42.0	64	1	64.0	1704.0	1825.4	380.4	305	303
4	+	48.2	80	3	51.6	1349.0	1555.0	313.0	298	277
5	-	63.0	58	2	69.6	1363.2	1776.2	398.0	275	270
6	+	13.5	64	1	53.2	1682.8	1326.0	300.0	311	298
7	-	11.5	47	1	48.4	1657.0	1324.4	288.4	290	295
42.9										
Mean		39.3	61.1	1.4	57.3	1714.9	1574.7	334.2	297.4	294.7
SEM		7.5	3.9	0.3	4.1	197.2	76.7	18.1	4.5	6.0
t-test		S _p <0.01	NS	NS	S _p <0.01	NS	S _p <0.001	S _p <0.001	NS	S _p <0.05

Table 13

Demonstrates the results obtained from expt. 3.

The oil was given orally in dosage of 291.3 mg/kg/day (equivalent to 100 gm fresh plant/kg/day) for continuous 15 days.

It could be seen than total sperm count was statistical significantly decreased in experimented animals as compared to control animals ($p < 0.01$) weight of cauda epidimymis was also decrease ($p < 0.01$) and there was highly significant ($p < 0.001$) decreased in weights of seminal vesicle and total prostate and ventral prostate. Although weight of testis was not altered, it should be noted that there water retention in testis of experiment animal No. 2, it was pale in color which was different from testis of control animals. There was also statistical significant difference in body weight gain ($p < 0.05$) during experimental period.

Table 14

No. of rat	Mating	Total sperm count (mill)	Sperm motility		Weight (mg)				Body wt. (gm)	
			%	grade	cauda epid.	testes	SV& total prost.	VP	start	end
<u>Control</u>										
1	+	66.8	79	3	82	1674	1956.0	474.0	274	320
2	+	65.5	79	3	78	1764	2468.8	480.8	299	361
3	+	49.2	61	1	81	1684	2630.6	540.0	290	352
4	+	46.5	65	2	60	1544	1744.8	444.8	265	309
5	+	68.8	73	2	70	1656	2142.0	510.0	296	342
6	+	59.0	67	2	69	1758	2519.0	502.0	308	363
100%										
Mean		59.3	70.7	2.2	73.3	1680	2243.5	491.9	288.7	341.2
SEM		3.9	3.1	0.3	3.5	32.8	143.6	13.4	6.6	9.1
<u>Experiment</u>										
1	+	64.3	61	1	62	1563	2221	311	312	301
2	+	66.8	77	1	78	1776	2076	370	330	313
3	-	40.8	72	1	46	1358	1619	365	277	264
4	+	4.0	41	1	42	964	1250	308	310	336
5	-	33.5	65	1	46	1694	2014	410	315	332
6	-	48.5	58	1	51	1552	1468	382	260	280
50%										
Mean		42.9	62.3	1	54.2	1484.5	1774.7	357.7	300.7	304.3
SEM		9.4	5.1	0	5.5	119.3	157.2	16.4	10.8	11.6
t-test		NS	NS	S _p <0.01	S _p <0.02	NS	NS	S _p <0.001	NS	S _p <0.05

Table 14

Demonstrates the results obtained from expt. 3. The volatile oil of sweet basil was given orally in dosage of 291.3 mg/kg/day (equivalent to 100 gm fresh plant/kg/day) for continuous 15 days. There was statistical significant reduction in weight of cauda epididymis ($p < 0.02$), in weight of ventral prostate ($p < 0.001$) and grade of motility ($p < 0.01$). However, testicular weights were not significantly changed. Body weight gain during experimental period was significantly decreased ($p < 0.05$). A 50% reduction in percentage of mating positive in experimental group was observed.

Table 15

No. of rats	Mating	Total sperm count (mill)	Sperm motility		Weight (mg)			Body wt.(gm)		
			% grade	cauda epid.	testes	SV& total prost.	VP	start	end	
<u>Control</u>										
1	-	85.7	73	3	81	1754	2830	632	337	335
2	+	62.0	43	1	72	1531	2178	463	336	337
3	+	105.5	75	2	89	1980	3003	620	354	370
4	+	50.0	46	1	59.6	1632	2793	569	335	343
75%										
Mean		75.8	59.2	1.7	75.4	1724.2	2701	571	340.5	346.2
SEM		12.4	8.5	0.5	6.3	48.3	180.2	38.5	4.5	8.1
<u>Experiment</u>										
1	-	2.8	0	0	38.0	2115	379	159	333	306
2	+	29.0	67	1	37.8	2640	1838	390	265	260
3	+	61.5	65	2	59.0	2617	2032	357	299	302
4	-	29.7	57	1	40.0	2012	1124	236	299	287
5	+	28.8	45	1	54.0	2160	1433	284	311	300
6	+	39.2	46	1	51.4	2204	1567	335	288	274
7	+	83.0	63	3	64.0	2534	1933	400	343	309
71.4%										
Mean		39.1	49	1.3	49.2	2326	1472.3	308.7	305.4	291.1
SEM		9.8	8.8	0.4	4.0	99	217.4	33.2	10.0	6.9
t-test		S _p <0.05	NS	NS	S _p <0.01	S _p <0.01	S _p <0.01	S _p <0.001	S _p <0.05	S _p <0.001

Table 15

Demonstrates the results obtained from expt. 3. The volatile oil of sweet basil was given orally in dosage of 582.6 mg/kg/day (equivalent to 200 gm of fresh plant/kg/day) for continuous 15 days. There was significant reduction in sperm concentration ($p < 0.05$), in weight of cauda epididymis ($p < 0.01$), in weight of seminal vesicle and prostate ($p < 0.01$) and of ventral prostate ($p < 0.01$). In this expt., weight of testis was significantly increased ($p < 0.01$) due to water retention in this organ. Body weight at the end of experiment was statistical highly significant decreased ($p < 0.001$). 75%, 71.4% of mating positive was observed in control and experiment groups respectively.

Table 16

No. of rats	Total sperm count (mill)	Sperm motility			Weight (mg)			Body wt. (gm)	
		%	grade	cauda epid.	testes	SV& total prost.	VP	start	end
<u>Control</u>									
1	72.5	64	1	62	1466	2098	470	325	359
2	80.0	80	2	82	1554	2448	489	293	337
3	94.5	73	3	92	1685	2584	540	323	368
4	92.5	69	1	92	1694	3292	754	304	336
5	35.7	47	1	58	1612	2086	421	300	335
6	60.5	75	3	92	1870	2726	650	320	355
Mean	72.6	68	1.8	79.7	1646.8	2539	554	310.8	348.3
SEM	9.0	4.7	0.4	6.4	56.6	183.7	51.1	5.5	5.8
<u>Experiment</u>									
1	21.6	21	1	43.0	2018	1782	162	306	326
2	14.3	0	0	28.2	1435	767	217	323	252
3	14.5	37	1	44.0	2267	1120	300	284	280
4	41.3	73	1	44.0	1819	1944	358	306	311
5	20.5	45	1	42.0	1710	1894	320	296	270
Mean	22.4	35.2	0.8	40.2	1849.8	1501.4	271.4	303	287.8
SEM	4.9	12.2	0.2	3.0	140.5	235.9	35.8	6.4	13.5
t-test	S _p < 0.01	S _p < 0.05	S _p < 0.05	S _p < 0.001	NS	S _p < 0.01	S _p < 0.01	NS	S _p < 0.01

Table 16

Demonstrates the results obtained from expt. 3. The volatile oil of sweet basil was given orally in dosage of 582.6 mg/kg/day (equivalent to 200 gm fresh plant/kg/day) for continuous 40 days. There was statistical significant decrease in sperm concentration ($p < 0.001$), in weight of cauda epididymis ($p < 0.001$), in weight of total prostate & seminal vesicle ($p < 0.01$) and in weight of ventral prostate ($p < 0.01$). Sperm motility was also reduced in both percentage ($p < 0.05$) and grade ($p < 0.05$). Weight of testis was not altered, however, there was obvious water retention in the testis of experiment animals No. 1 and No. 3. A reduction in body weight gain was also significant evident ($p < 0.01$)

Originally it was planned to feed for 60 days continuously but due to toxicity manifestation of the testing material, experimental period was stopped at 40 days and mating was not performed.

Table 17

No. of rats	Mating	Total sperm count (mill)	Sperm motility		Weight (mg)				Body wt. (gm)	
			%	grade	cauda epid.	testes	SV& total prost.	VP	start	end
<u>Control</u>										
1	+	153.7	38	3	236	1563	3253	616	400	420
2	+	165.9	79	3	240	1903	3394	548	390	395
3	+	113.2	74	2	204	1636	2652	576	342	365
4	+	126.0	80	2	202	1571	2762	512	350	398
5	+	142.5	69	2	201	1776	2843	528	338	354
6	+	100.1	80	2	216	1990	2462	596	373	409
7	+	159.7	81	3	252	1783	3604	520	387	404
100%										
Mean		137.3	78.7	2.4	221.6	1746	2995.7	556.6	368.6	392.1
SEM		9.4	2.2	0.2	7.9	62.2	160.0	15.2	9.5	9.1
<u>Experiment</u>										
1	+	100.5	70	2	227	3570	2578	412	388	375
2	+	97.1	68	2	150	1690	2621	575	350	337
3	+	108.0	69	2	195.8	1533	2178	398	369	360
4	+	107.2	72	3	172	2578	2094	500	368	378
5	+	66.4	80	2	164	2046	1122	441	330	300
6	+	82.5	65	3	184	3160	1686	496	384	398
7	-	73.1	59	2	194	3098	1475	339	325	298
85.7%										
Mean		90.7	69	2.3	183.8	2525	1964.9	451.6	359.1	349.4
SEM		6.3	2.4	0.2	9.5	298.3	212.5	29.6	9.4	14.8
t-test		S _p <0.01	S _p <0.02	NS	S _p <0.01	S _p <0.05	S _p <0.01	S _p <0.01	NS	S _p <0.05

Table 17

Demonstrates the results obtained from expt. 3. The volatile oil of sweet basil was orally given in dosage of 320 mg/kg/day for continuous 60 days. There was statistical significant reduction in sperm concentration ($p < 0.01$) and percentage of sperm motility ($p < 0.02$), weight of cauda epididymis ($p < 0.01$), weight of total prostate & seminal vesicle ($p < 0.01$) and of ventral prostate. For testicular weight, it was significantly increased ($p < 0.05$) due to water retention. Body weight gain was also statistical significant different ($p < 0.05$). 100%, 85.7% of mating positive was observed in control and experiment groups respectively.

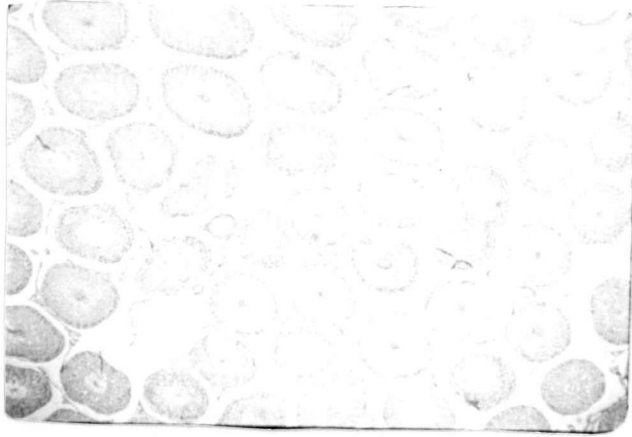


Table 18 Summarized data of antifertility study of oil of O. basilicum in rats.

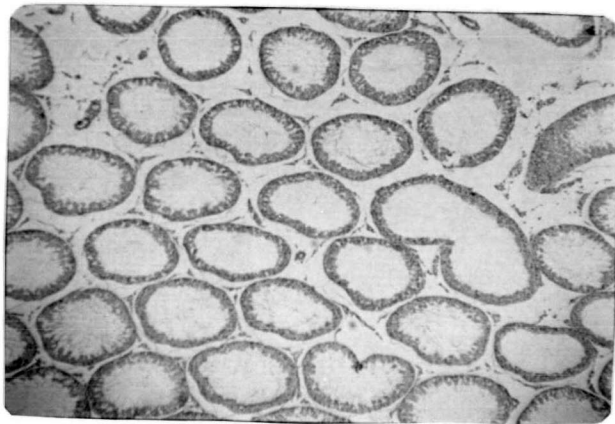
Dose Day	145.6mg/kg/day		291.3mg/kg/day		582.6mg/kg/day		320mg/kg/day	
	15 %	60 %	15 %	60 %	15 %	40 %	15 %	60 %
Sperm count	NS	NS	↓56.5	NS	↓48.4	↓59.1	-	↓33.9
Motility								
percent	NS	NS	NS	NS	NS	↓48.2	-	↓12.3
grade	NS	NS	NS	↓54.5	NS	↓55.6	-	NS
Weight of								
caud.epid.	NS	NS	↓26.7	↓16.1	↓34.7	↓49.6	-	↓17.0
testis	NS	NS	NS	NS	↑34.9	NS	-	↑44.6
SV&prost.	NS	NS	↓33.6	NS	↓45.5	↓40.9	-	↓34.3
VP	NS	NS	↓31.9	↓27.3	↓45.9	↓51.0	-	↓18.8
Body wt.	NS	NS	NS	C ↑18.2 E ↑1.2	-	C ↑11 E ↓5	-	C ↑6.4 E ↓2.8
Mating	no change	↓50	no change	↓50	no change	-	-	↓14.3

Result of histological study

Histological examination of the testes of the rat given oil of O. basilicum indose of 320 mg/kg/day orally & daily for 60 days showed a significant changes in spermatogenic elements in the seminiferous tubules (figure 24, 25 p. 115, 116) of the treated animals. Approximately, 90 % of seminiferous tubules were devoid of spermatids and spermatozoa & only spermatogonia were found. Seminiferous tubules were enlarge in size as compared to those of control group. There was also a decrease in number of Sertoli cell but cellular characters are still normal. Leydig cell appeared normal. Mutagenic changes were not observed.

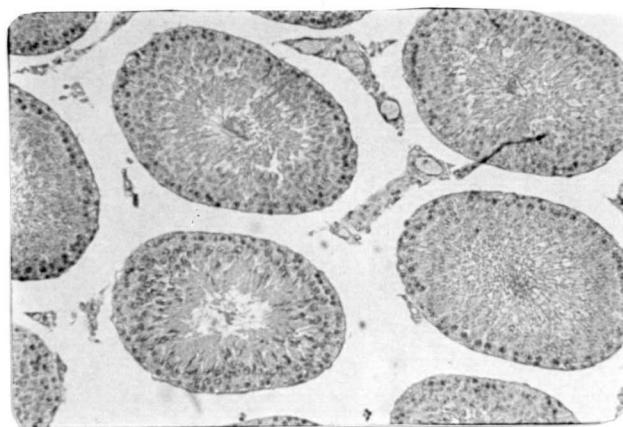


(1)

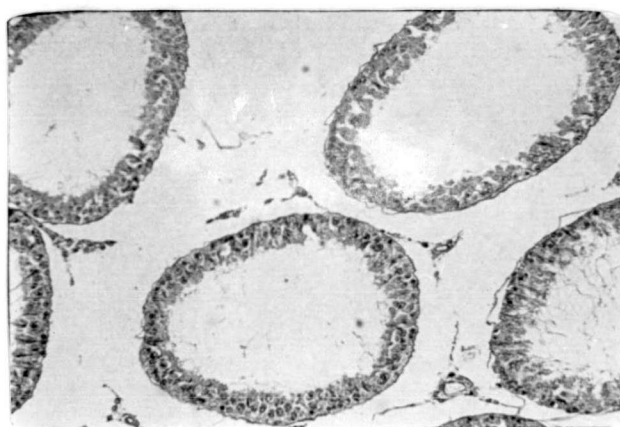


(2)

Figure 25 The photographs illustrating testicular histology of rat (1) control animals (2) treated animals with oil of *O. basilicum* X 100 magnification.



(1)



(2)

Figure 25 The photographs illustrating testicular histology of rat (1) control animals (2) treated animals with oil of *O.basilicum* X 400 magnification.

Table 19

Demonstrates the results obtained from expt 4. The volatile oil of O. sanctum was given orally and daily in dose of 92.3 mg/kg/day (equivalent to 100 gm fresh plant/kg/day) for a period of 15 days. There was no statistical significant difference in all of the parameters measured. Percentage of mating positive animals was not reduced in experimental group.

Table 20

Demonstrates the results obtained from expt. 4. Oil of O. sanctum was given orally and daily in dose of 92.3 mg/kg/day. (equivalent to 100 gm fresh plant/kg/day) for a period of 60 days. There was no statistical significant difference in all parameters measured. Percentage of mating positive animals was not reduced in experimental group.

Table 21

Demonstrates the results obtained from expt. 4. Oil of O. sanctum was given orally and daily in dose of 184.6 mg/kg/day (equivalent to 200 gm fresh plant/kg/day) for a period of 15 days. There was no statistical significant difference in all of the parameters measured. Percentage of mating positive animals was not reduced in experimental group.

Table 22 :

Demonstrates the results obtained from expt. 4. Oil of O. sanctum was given orally and daily in dose of 184.6 mg/kg/day (equivalent to 200 gm fresh plant/kg/day for a period of 60 days. There was no statistical significant difference in all of the parameters measured. Percentage of mating positive animals was not reduced in experimental group.