# CHAPTER III
# MATERIALS AND METHODS

## 3.1 Experimental animals

Female Wistar rats with regular estrous cycle ( 4-5 days ) for at least 3 consecutive cycles, age 60 days, weighting 200-250 gm, used in this study were supplied from National Laboratory Animal Centre, Mahidol University. The rats were housed at the Animal's Laboratory House, Primate Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University. Five rats were housed in a stainless steel cage, 24.5 x 46 x 4 cm. of size, throughout an environment-controlled room with 25-26 °C and a 12-hour light/12-hour dark cycle. The animals were fed with rat chow diet (Pokapan Animal Food Center, Thailand) and tap water *ad libitum*.

## 3.2 Rat chow diet

Animal chow diet was purchased from Pokapan animal Food Centre, Thailand. Ingredients of the diet were shown in Table 3.1.

**Table 3.1**   Ingredients of the rat chow diet

| *Product specification* | | |
|---|---|---|
| Product name: | C.P.082 | |
| Description: | Rat and mice food | |
| Ingredients: | Yellow corn, soybean meal, full-fat soybean, rice, rice by-product, fish meal, corn gluten meal, vegetable oil, salt, vitamins and minerals | |
| Nutrients: | Moisture | <10.0% |
| | Protein | >24.0% |
| | Fat | >4.5% |
| | Fiber | <5.0% |
| | Calcium | >0.9% |
| | Phosphorus | >0.7% |

### 3.3 Plant materials

The tuberous roots of cultivated *P. mirifica* (PM-III and PM-IV) were collected from a farm at Ratchaburi province. To minimize the environmental factors variation of phytoestrogens content in *P. mirifica*, the tuberous roots used in this study were collected in 12 months. (From March 2005 to February 2006).

The collected tubers were cleaned, sliced, dried in a hot air oven at 70°C until nearly completely dried and subsequently ground into powder at a size of 100 Mesh (Cherdshewasart, 2004[a]).

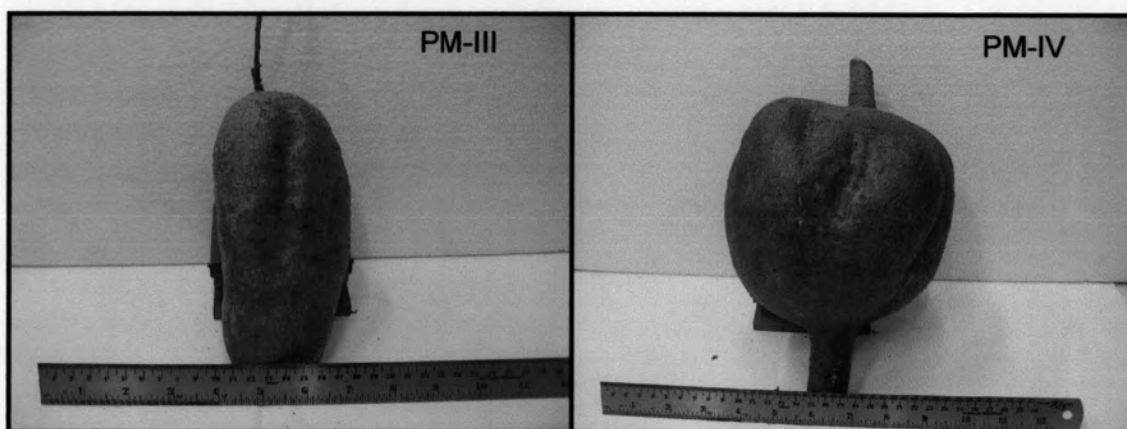### 3.4 Morphometry of PM-III and PM-IV

### 3.4.1 Tuber



**Figure 3.1**  The tuber of PM-III and PM-IV

### 3.4.1.1 Size of tuber

Sizes of tuber (circumference, height, width) of PM-III and PM-IV were shown in Table 3.2.

**Table 3.2** Sizes of tuber

| NO. | PM-III | | | PM-IV | | |
|---|---|---|---|---|---|---|
| | circumference (cm) | height (cm) | width (cm) | circumference (cm) | height (cm) | width (cm) |
| 1 | 19.50 | 17.00 | 7.00 | 50.50 | 18.00 | 17.00 |
| 2 | 27.00 | 19.50 | 9.00 | 37.00 | 8.00 | 12.5.00 |
| 3 | 21.00 | 15.00 | 8.50 | 36.00 | 7.00 | 11.50 |
| 4 | 24.00 | 18.00 | 6.00 | 23.50 | 10.00 | 7.50 |
| 5 | 30.00 | 16.00 | 3.50 | 55.00 | 17.00 | 17.50 |
| 6 | 41.00 | 40.00 | 13.00 | 25.50 | 12.00 | 8.50 |
| 7 | 27.00 | 15.50 | 8.50 | 27.50 | 11.50 | 12.00 |
| 8 | 31.00 | 16.00 | 10.00 | 28.50 | 8.00 | 15.00 |
| 9 | 25.00 | 20.00 | 13.00 | 35.00 | 9.00 | 15.00 |
| 10 | 16.50 | 16.00 | 10.00 | 55.00 | 17.00 | 20.50 |
| 11 | 20.00 | 25.00 | 10.50 | 45.00 | 12.00 | 16.00 |
| 12 | 24.00 | 22.00 | 9.00 | 39.00 | 8.00 | 15.50 |
| mean | 25.50* | 20.00* | 9.00 | 38.13* | 11.46 | 14.04 |
| S.E.M | 25.50 | 20.00 | 9.00 | 38.12 | 11.45 | 14.18 |

* The mean difference compared with width at $P<0.05$

## 3.4.1.2 The tuber weight

The tuber weight and percentage of water in the tuber of PM-III and PM-IV were shown in Table 3.3.

**Table 3.3** Weights of tuber

| Month | PM-III | | | PM-IV | | |
|---|---|---|---|---|---|---|
| | wet weight (g) | dry weight (g) | % water in tuber | wet weight (g) | dry weight (g) | % water in tuber |
| March | 4200.00 | 287.92 | 93.14 | 2400.00 | 193.89 | 91.92 |
| | 2200.00 | 207.10 | 90.49 | 3200.00 | 301.10 | 90.53 |
| | 1900.00 | 153.95 | 91.90 | 1300.00 | 122.83 | 90.55 |
| mean | 2766.67±721.88 | 216.32±38.95 | 91.88±0.77 | 2300.00±550.76 | 206.61±51.81 | 91.00±0.46 |
| April | 2732.00 | 352.00 | 87.12 | 7583.00 | 625.00 | 91.76 |
| | 2928.00 | 304.00 | 89.62 | 3220.00 | 323.00 | 89.97 |
| | 4088.00 | 446.00 | 89.09 | 5480.00 | 516.00 | 90.58 |
| mean | 3249.33±423.13 | 367.33±41.70 | 88.61±0.76 | 5427.67±1259.76 | 488.00±88.30 | 90.77±0.53 |
| May | 7961.00 | 713.00 | 91.04 | 2874.00 | 277.00 | 90.36 |
| | 2307.00 | 241.00 | 89.55 | 1778.00 | 177.00 | 90.04 |
| | 2182.00 | 270.00 | 87.63 | 4351.00 | 452.00 | 89.61 |
| mean | 4150.00±1905.84 | 408.00±152.73 | 89.41±0.99 | 3001.00±745.47 | 302.00±80.36 | 90.01±0.22 |
| June | 741.00 | 76.00 | 89.74 | 2643.00 | 217.00 | 91.79 |
| | 3155.00 | 307.00 | 90.27 | 2229.00 | 236.00 | 89.41 |
| | 1084.00 | 101.00 | 90.68 | 841.00 | 85.00 | 89.89 |
| mean | 1660.00±754.03 | 161.33±73.19 | 90.23±0.27 | 1904.33±544.93 | 179.33±47.48 | 90.36±0.73 |

**Table 3.3** Weights of tuber (continued)

| Month | PM-III | | | PM-IV | | |
|---|---|---|---|---|---|---|
| | wet weight (g) | dry weight (g) | % water in tuber | wet weight (g) | dry weight (g) | % water in tuber |
| July | 1359.00 | 101.00 | 92.57 | 1986.00 | 170.00 | 91.44 |
| | 755.00 | 66.00 | 91.26 | 2083.00 | 163.00 | 92.17 |
| | 1492.00 | 134.00 | 91.02 | 2553.00 | 219.00 | 91.42 |
| mean | 1202.00±226.77 | 100.33±19.63 | 91.62±0.48 | 2207.33±175.09 | 184.00±17.62 | 91.68±0.25 |
| August | 3025.00 | 352.00 | 88.36 | 4600.00 | 465.00 | 89.89 |
| | 1180.00 | 96.00 | 91.86 | 3250.00 | 353.00 | 89.14 |
| | 720.00 | 59.00 | 91.81 | 3880.00 | 350.00 | 90.98 |
| mean | 1641.67±704.30 | 169.00±92.12 | 90.68±1.16 | 3910.00±390.00 | 389.33±37.84 | 90.00±0.53 |
| September | 1900.00 | 127.00 | 93.32 | 4000.00 | 278.00 | 93.05 |
| | 800.00 | 79.00 | 90.13 | 3700.00 | 365.00 | 90.14 |
| | 600.00 | 63.00 | 89.50 | 4200.00 | 431.00 | 89.74 |
| mean | 1100.00±404.15 | 89.67±19.23 | 90.98±1.18 | 3966.67±145.30 | 358.00±44.31 | 90.97±1.04 |
| October | 334.00 | 19.00 | 94.31 | 3500.00 | 341.00 | 90.26 |
| | 1900.00 | 248.00 | 86.95 | 1300.00 | 134.00 | 89.69 |
| | 700.00 | 73.00 | 89.57 | 1500.00 | 174.00 | 88.40 |
| mean | 978.00±472.95 | 113.33±69.11 | 90.28±2.15 | 2100.00±702.38 | 216.33±63.39 | 89.45±0.55 |

**Table 3.3** Weights of tuber (continued)

| Month | PM-III | | | PM-IV | | |
|---|---|---|---|---|---|---|
| | wet weight (g) | dry weight (g) | % water in tuber | wet weight (g) | dry weight (g) | % water in tuber |
| November | 7800.00 | 605.00 | 92.24 | 900.00 | 95.00 | 89.44 |
| | 600.00 | 37.00 | 93.83 | 2200.00 | 37.00 | 98.32 |
| | 400.00 | 50.00 | 87.50 | 2500.00 | 231.00 | 90.76 |
| mean | 2933.33±2434.02 | 230.67±187.20 | 91.19±1.90 | 1866.67±491.03 | 121.00±57.49 | 92.84±2.77 |
| December | 1854.00 | 109.00 | 94.12 | 1380.00 | 195.00 | 85.87 |
| | 1645.00 | 250.00 | 84.80 | 5379.00 | 410.00 | 92.38 |
| | 1003.00 | 140.00 | 86.04 | 2000.00 | 223.00 | 88.85 |
| mean | 1500.67±256.04 | 166.33±42.78 | 88.32±2.92 | 2919.67±1242.62 | 276.00±67.49 | 89.03±1.88 |
| January | 1100.00 | 96.00 | 91.27 | 1200.00 | 166.00 | 86.17 |
| | 1200.00 | 201.00 | 83.25 | 3100.00 | 372.00 | 88.00 |
| | 1800.00 | 145.00 | 91.94 | 1300.00 | 135.00 | 89.62 |
| mean | 1366.67±218.58 | 147.33±30.33 | 88.82±2.79 | 1866.67±617.34 | 224.33±74.37 | 87.93±1.00 |
| February | 2200.00 | 207.00 | 90.59 | 2400.00 | 194.00 | 91.92 |
| | 1900.00 | 154.00 | 91.89 | 1300.00 | 123.00 | 90.54 |
| | 2050.00 | 180.50 | 91.20 | 1850.00 | 158.50 | 91.43 |
| mean | 2050.00±86.60 | 180.50±15.30 | 91.23±0.38 | 1850.00±317.54 | 158.5±20.50 | 91.30 0.40 |

## 3.4.2 Leafs
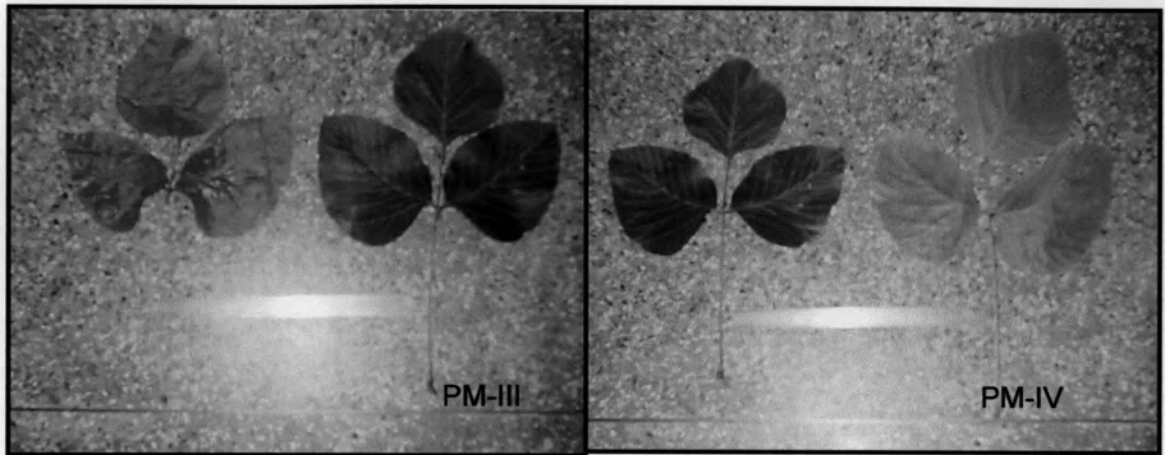


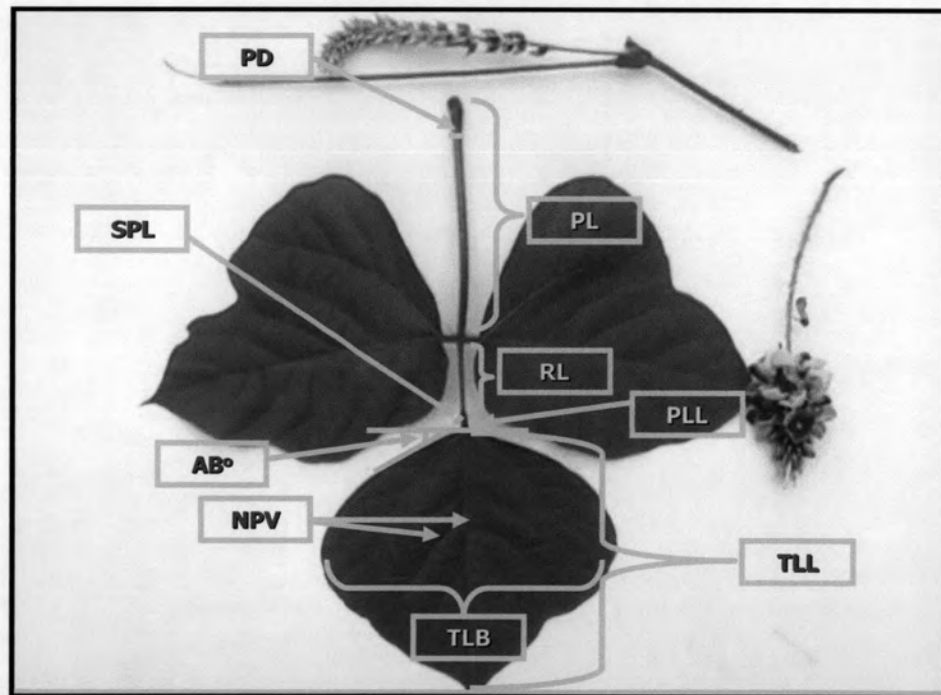**Figure 3.2**  The leaf of PM-III and PM-IV



**Figure 3.3**   Morphometry parameters of *P. mirifica* leaf. (petiole length – PL, petiole diameter – PD, rachis length – RL, petiolet length – PLL, terminal leaflet length – TLL, terminal leaflet breadth – TLB, stipule length – SPL, angle of first leaf border – $(A\char"005EB)^\circ$, number of pairs of primary veins – NPV

**Table 3.4** Leaf morphometry of PM-III

| NO. | PL(cm) | PD(cm) | RL(cm) | PLL(cm) | TLL(cm) | TLB(cm) | SPL(cm) | A^B(°) | NPV(cm) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 28.8 | 0.48 | 6.5 | 0.97 | 23 | 18.2 | 0.37 | 23 | 7.5 |
| 2 | 44.8 | 0.43 | 7.6 | 0.74 | 25 | 18 | 0.48 | 30 | 8 |
| 3 | 17.8 | 0.3 | 4.9 | 1.34 | 19.3 | 13.8 | 0.35 | 29 | 6.5 |
| 4 | 21 | 0.34 | 5.7 | 1.35 | 21.6 | 16.4 | 0.41 | 22 | 8 |
| 5 | 26 | 0.35 | 5.6 | 1.24 | 18 | 13.8 | 0.34 | 19 | 7.5 |
| 6 | 27.5 | 0.32 | 6.5 | 1 | 23.5 | 16.5 | 0.4 | 19 | 6 |
| 7 | 29 | 0.34 | 5.2 | 1.14 | 24.2 | 18 | 0.4 | 27 | 7.5 |
| 8 | 18.5 | 0.33 | 3.5 | 1.13 | 19.5 | 12.8 | 0.39 | 21 | 6 |
| 9 | 20.3 | 0.39 | 7 | 1.22 | 21.8 | 16.6 | 0.44 | 29 | 6.5 |
| 10 | 17.5 | 0.27 | 3.4 | 0.7 | 18.5 | 11.5 | 0.33 | 42 | 6 |
| 11 | 17.3 | 0.3 | 3.8 | 0.96 | 18.5 | 11 | 0.3 | 41 | 6 |
| 12 | 22.6 | 0.27 | 5.1 | 0.74 | 19.2 | 14 | 0.33 | 33 | 8 |
| 13 | 19.3 | 0.27 | 5 | 0.83 | 19.8 | 14.7 | 0.3 | 32 | 7 |
| 14 | 36.7 | 0.42 | 7.7 | 0.91 | 21.7 | 17.2 | 0.4 | 21 | 6 |
| 15 | 21.7 | 0.27 | 4.1 | 0.98 | 19.9 | 14.7 | 0.33 | 42 | 7.5 |
| 16 | 22.5 | 0.33 | 4.7 | 0.94 | 18.6 | 12.7 | 0.38 | 37 | 6 |
| 17 | 15.7 | 0.33 | 5 | 0.88 | 25.8 | 18.9 | 0.44 | 27 | 7 |
| 18 | 26.6 | 0.33 | 6.6 | 0.77 | 24.3 | 18.2 | 0.4 | 28 | 7 |
| 19 | 34.2 | 0.44 | 8 | 0.74 | 24 | 22.5 | 0.46 | 13 | 7 |
| 20 | 28.3 | 0.35 | 5.9 | 1.05 | 23.3 | 19.7 | 0.39 | 26 | 6.5 |
| 21 | 14.6 | 0.27 | 4.3 | 0.85 | 26 | 18.4 | 0.46 | 30 | 6 |
| 22 | 27.5 | 0.33 | 4.7 | 0.94 | 26.5 | 18.2 | 0.44 | 31 | 6.5 |
| 23 | 18.5 | 0.36 | 6 | 1.1 | 28.5 | 19.8 | 0.44 | 41 | 6 |
| 24 | 27.9 | 0.33 | 4.8 | 0.88 | 21.6 | 16.4 | 0.36 | 21 | 6.5 |
| 25 | 28.3 | 0.31 | 6.5 | 0.85 | 20 | 16 | 0.3 | 20 | 6 |
| 26 | 22.7 | 0.41 | 5.5 | 0.91 | 19.5 | 16 | 0.46 | 24 | 6.5 |
| 27 | 32.2 | 0.33 | 5.5 | 0.85 | 21.7 | 15.3 | 0.34 | 33 | 6.5 |
| 28 | 17.7 | 0.26 | 3.5 | 0.95 | 17.9 | 11.6 | 0.32 | 39.5 | 5.5 |
| 29 | 17.3 | 0.28 | 4.3 | 0.94 | 22.3 | 14 | 0.37 | 40 | 6 |

**Table 3.4** Leaf morphometry of PM-III (continued)

| NO. | PL(cm) | PD(cm) | RL(cm) | PLL(cm) | TLL(cm) | TLB(cm) | SPL(cm) | A^B(°) | NPV(cm) |
|-----|--------|--------|--------|---------|---------|---------|---------|--------|---------|
| 30 | 16.7 | 0.29 | 3.2 | 0.83 | 22 | 14.8 | 0.36 | 41 | 6.5 |
| 31 | 18.4 | 0.4 | 4.2 | 1.3 | 20.5 | 16 | 0.44 | 26 | 6 |
| 32 | 29.5 | 0.36 | 5.4 | 1.05 | 19 | 16.5 | 0.36 | 30 | 5.5 |
| 33 | 21.2 | 0.36 | 5.5 | 1.13 | 22 | 16.2 | 0.44 | 31 | 7 |
| 34 | 32.9 | 0.36 | 5 | 1.13 | 21 | 15.1 | 0.41 | 33 | 6 |
| 35 | 17.7 | 0.34 | 4.3 | 1.05 | 23.2 | 17.2 | 0.42 | 22 | 6 |
| 36 | 35.8 | 0.31 | 6.5 | 1.16 | 21 | 14.5 | 0.35 | 28 | 6 |
| 37 | 26.5 | 0.34 | 5.2 | 1.07 | 21.5 | 16.4 | 0.43 | 24 | 7.5 |
| 38 | 23.3 | 0.42 | 5 | 1.27 | 19.7 | 16.2 | 0.39 | 24 | 6.5 |
| 39 | 25.4 | 0.26 | 5.1 | 0.76 | 20.7 | 15.9 | 0.33 | 30 | 7 |
| 40 | 18.5 | 0.24 | 4 | 0.99 | 19.3 | 13.5 | 0.33 | 40 | 7 |
| 41 | 16.5 | 0.3 | 3.4 | 0.97 | 25 | 18.3 | 0.4 | 20 | 7 |
| 42 | 44.5 | 0.36 | 6.8 | 1.16 | 21.6 | 14.6 | 0.34 | 24 | 7.5 |
| 43 | 13.8 | 0.24 | 4.1 | 0.6 | 15.7 | 9.5 | 0.3 | 53 | 6 |
| 44 | 37.7 | 0.35 | 5.4 | 0.72 | 20.9 | 15.1 | 0.3 | 25 | 7.5 |
| 45 | 31.3 | 0.36 | 5.4 | 1.72 | 22 | 16.6 | 0.4 | 34 | 6 |
| 46 | 32.5 | 0.4 | 6.65 | 0.95 | 21.2 | 16.25 | 0.25 | 33 | 8.5 |
| 47 | 44.55 | 0.45 | 8.95 | 0.85 | 21.3 | 17.85 | 0.25 | 35 | 9.5 |
| 48 | 40.8 | 0.55 | 8.75 | 0.95 | 21.3 | 18.7 | 0.2 | 35 | 10 |
| 49 | 26 | 0.45 | 6.55 | 1.05 | 25.1 | 22.05 | 0.53 | 23.5 | 6.5 |
| 50 | 31 | 0.35 | 6.05 | 0.9 | 19.05 | 13.6 | 0.15 | 39.5 | 8 |
| MEAN | 25.75[e] | 0.35[a] | 5.45[b] | 0.995[a] | 21.53[d] | 15.99[c] | 0.37[a] | 29.83[f] | 6.81[b] |
| S.E.M. | 1.16 | 0.01 | 0.19 | 0.03 | 0.36 | 0.37 | 0.01 | 1.13 | 0.13 |

Means for groups in homogeneous subsets are displayed

**Table 3.5**  Leaf morphometry of PM-IV

| NO. | PL(cm) | PD(cm) | RL(cm) | PLL(cm) | TLL(cm) | TLB(cm) | SPL(cm) | A^B(°) | NPV(cm) |
|-----|--------|--------|--------|---------|---------|---------|---------|--------|---------|
| 1 | 38.5 | 0.56 | 8.6 | 1.06 | 30.4 | 27.6 | 0.28 | 7 | 7 |
| 2 | 35.1 | 0.43 | 7.1 | 0.96 | 22 | 18.3 | 0.29 | 19 | 8 |
| 3 | 42.6 | 0.44 | 8.3 | 0.98 | 24.2 | 19.7 | 0.37 | 18 | 9 |
| 4 | 32.8 | 0.43 | 7.7 | 0.95 | 22.6 | 17.6 | 0.38 | 17 | 9.5 |
| 5 | 31 | 0.46 | 7.7 | 0.98 | 24.2 | 19.3 | 0.36 | 23 | 7.5 |
| 6 | 27.2 | 0.38 | 7.1 | 0.9 | 23.4 | 18.7 | 0.42 | 30 | 8.5 |
| 7 | 33.3 | 0.37 | 7 | 0.98 | 21.6 | 18.4 | 0.44 | 27 | 8 |
| 8 | 38.1 | 0.38 | 7.1 | 0.89 | 20.7 | 17.3 | 0.46 | 26 | 8 |
| 9 | 31.7 | 0.48 | 6.4 | 0.79 | 25.5 | 20.4 | 0.28 | 32 | 8 |
| 10 | 33.2 | 0.47 | 7.4 | 1.05 | 27.4 | 21.6 | 0.45 | 30 | 8 |
| 11 | 25.1 | 0.42 | 7.3 | 1.04 | 26.8 | 19.8 | 0.51 | 27 | 7 |
| 12 | 30.3 | 0.4 | 9.6 | 1.04 | 29.1 | 19 | 0.45 | 31 | 7 |
| 13 | 36.7 | 0.4 | 7.6 | 1.02 | 30 | 22.4 | 0.36 | 24 | 7.5 |
| 14 | 32.2 | 0.41 | 6.2 | 0.95 | 25.1 | 24.2 | 0.32 | 13 | 7.5 |
| 15 | 26.8 | 0.39 | 9 | 0.95 | 32.2 | 20.7 | 0.32 | 18 | 8.5 |
| 16 | 32.7 | 0.43 | 8.6 | 1.2 | 29 | 25.7 | 0.3 | 10 | 8.5 |
| 17 | 28.2 | 0.5 | 10.5 | 1.15 | 24.2 | 21.5 | 0.41 | 17 | 8 |
| 18 | 36 | 0.45 | 9.3 | 1.1 | 28.4 | 20.8 | 0.35 | 23 | 8 |
| 19 | 25.2 | 0.49 | 9.4 | 1.02 | 23 | 22 | 0.45 | 23 | 9 |
| 20 | 32.1 | 0.43 | 8.7 | 0.97 | 24.2 | 21.5 | 0.48 | 20 | 8 |
| 21 | 33.5 | 0.49 | 9.1 | 0.88 | 27.4 | 22.3 | 0.47 | 24 | 8 |
| 22 | 29.5 | 0.51 | 7.7 | 0.87 | 26.1 | 27.4 | 0.42 | 20 | 11 |
| 23 | 27.4 | 0.4 | 7 | 0.98 | 25.8 | 19.5 | 0.41 | 32 | 8 |
| 24 | 30.62 | 0.45 | 6.9 | 0.96 | 27 | 24.8 | 0.37 | 18 | 8 |
| 25 | 30.24 | 0.52 | 6.8 | 0.95 | 23.8 | 26 | 0.32 | 22 | 8 |
| 26 | 36.5 | 0.47 | 7.2 | 1.21 | 25.6 | 23.5 | 0.45 | 14 | 8 |
| 27 | 31.6 | 0.5 | 6.9 | 1.05 | 24.8 | 27.1 | 0.53 | 12 | 8 |
| 28 | 34.4 | 0.39 | 6.8 | 0.92 | 24.6 | 23.4 | 0.38 | 24 | 8 |
| 29 | 27.1 | 0.44 | 8.6 | 0.89 | 22 | 16.1 | 0.49 | 34 | 8 |

**Table 3.5** Leaf morphometry of PM-IV (continued)

| NO. | PL(cm) | PD(cm) | RL(cm) | PLL(cm) | TLL(cm) | TLB(cm) | SPL(cm) | A^B(°) | NPV(cm) |
|---|---|---|---|---|---|---|---|---|---|
| 30 | 33.7 | 0.46 | 6.1 | 0.89 | 21 | 18.7 | 0.52 | 32 | 9 |
| 31 | 29.8 | 0.41 | 7.2 | 0.65 | 22.5 | 19.6 | 0.48 | 25 | 7 |
| 32 | 28.5 | 0.44 | 8.3 | 0.79 | 25.8 | 20.5 | 0.49 | 31 | 7.5 |
| 33 | 25.5 | 0.43 | 6.5 | 0.92 | 25.3 | 22.8 | 0.58 | 27 | 7.5 |
| 34 | 33.6 | 0.43 | 7 | 0.89 | 21.7 | 17.1 | 0.4 | 32 | 8 |
| 35 | 41.1 | 0.39 | 6.8 | 0.86 | 21.5 | 18.9 | 0.48 | 28 | 7.5 |
| 36 | 39.2 | 0.42 | 5.3 | 0.85 | 21.6 | 20 | 0.52 | 27 | 7.5 |
| 37 | 21.2 | 0.39 | 7.5 | 0.85 | 23.1 | 19.9 | 0.39 | 24 | 8.5 |
| 38 | 21.6 | 0.42 | 7.1 | 0.92 | 21 | 18.1 | 0.41 | 30 | 6.5 |
| 39 | 25.8 | 0.41 | 7.6 | 0.92 | 26.2 | 22.8 | 0.38 | 25 | 8 |
| 40 | 31.2 | 0.47 | 7.6 | 0.93 | 22.6 | 17.8 | 0.41 | 27 | 8 |
| 41 | 35.1 | 0.49 | 6.5 | 0.87 | 23.4 | 17.7 | 0.32 | 25 | 8.5 |
| 42 | 27.7 | 0.52 | 6.9 | 0.9 | 23.1 | 19.7 | 0.41 | 25 | 8 |
| 43 | 33.2 | 0.42 | 8.1 | 0.82 | 23.2 | 19.6 | 0.48 | 22 | 8 |
| 44 | 30.1 | 0.38 | 6.5 | 0.79 | 22.6 | 20.1 | 0.41 | 27 | 8 |
| 45 | 52.7 | 0.46 | 8.2 | 0.89 | 21.2 | 17.6 | 0.48 | 20 | 6.5 |
| 46 | 38.1 | 0.43 | 7.1 | 0.8 | 21.8 | 16.9 | 0.55 | 23 | 6 |
| 47 | 40.7 | 0.48 | 9.2 | 0.81 | 21.8 | 17.9 | 0.52 | 24 | 6 |
| 48 | 39.7 | 0.5 | 9.4 | 0.75 | 20.4 | 17.6 | 0.42 | 24 | 6.5 |
| 49 | 30.2 | 0.4 | 8.8 | 0.8 | 23.6 | 20.6 | 0.4 | 26 | 7 |
| 50 | 33.5 | 0.44 | 8.7 | 0.82 | 22.4 | 19.1 | 0.44 | 29 | 7 |
| MEAN | 32.44[e] | 0.44[a] | 7.68[b] | 0.93[a] | 24.34[d] | 20.59[c] | 0.42[a] | 23.76[d] | 7.84[b] |
| S.E.M. | 0.80 | 0.01 | 0.15 | 0.02 | 0.40 | 0.41 | 0.01 | 0.86 | 0.12 |

Means for groups in homogeneous subsets are displayed

### 3.4.3 Pods
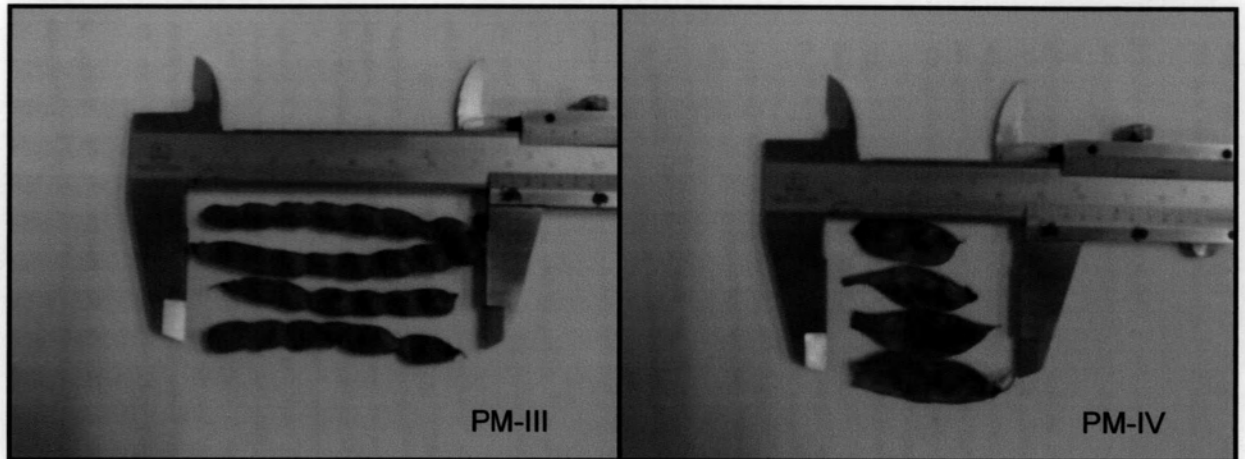


**Figure 3.4** Pods of PM-III and PM-IV

**Table 3.6**  Pod morphometry of PM-III and PM-IV

| No. | PM-III | | | PM-IV | | |
|---|---|---|---|---|---|---|
| | length(cm) | width(cm) | seed/pod | length(cm) | width(cm) | seed/pod |
| 1 | 7.40 | 0.70 | 8.00 | 4.46 | 0.93 | 3 |
| 2 | 7.20 | 0.80 | 6.00 | 4.59 | 0.86 | 2 |
| 3 | 7.00 | 0.75 | 7.00 | 3.93 | 0.48 | 5 |
| 4 | 7.20 | 0.80 | 7.00 | 4.48 | 1.17 | 3 |
| 5 | 5.90 | 0.80 | 5.00 | 5.49 | 0.79 | 4 |
| 6 | 5.30 | 1.00 | 4.00 | 3.93 | 0.76 | 3 |
| 7 | 5.90 | 0.75 | 6.00 | 4.16 | 0.68 | 4 |
| 8 | 5.90 | 0.80 | 6.00 | 4.09 | 0.75 | 4 |
| 9 | 6.00 | 0.85 | 5.00 | 4.55 | 0.76 | 3 |
| 10 | 5.60 | 0.85 | 5.00 | 3.56 | 0.87 | 2 |
| 11 | 6.10 | 0.90 | 6.00 | 4.19 | 0.69 | 4 |
| 12 | 5.60 | 1.00 | 6.00 | 3.79 | 0.9 | 2 |
| 13 | 6.50 | 0.90 | 7.00 | 4.86 | 0.66 | 3 |
| 14 | 6.80 | 0.90 | 7.00 | 3.7 | 0.7 | 3 |
| 15 | 7.10 | 1.00 | 8.00 | 4.63 | 0.63 | 3 |
| 16 | 7.50 | 0.90 | 8.00 | 5.27 | 0.79 | 4 |
| 17 | 8.60 | 1.00 | 6.00 | 4.07 | 0.63 | 4 |
| 18 | 5.70 | 0.85 | 6.00 | 3.75 | 0.81 | 2 |
| 19 | 6.00 | 0.95 | 6.00 | 6.09 | 0.94 | 5 |
| 20 | 7.40 | 1.00 | 8.00 | 4.87 | 0.8 | 4 |
| 21 | 6.50 | 0.90 | 7.00 | 7.47 | 0.89 | 5 |
| 22 | 5.50 | 1.00 | 6.00 | 4.35 | 1.18 | 3 |
| 23 | 5.90 | 0.90 | 5.00 | 4.29 | 0.96 | 3 |
| 24 | 6.70 | 0.90 | 7.00 | 4.65 | 0.77 | 4 |
| 25 | 5.20 | 0.80 | 6.00 | 5.27 | 0.7 | 3 |
| 26 | 5.60 | 0.90 | 6.00 | 3.46 | 0.79 | 2 |
| 27 | 6.20 | 0.95 | 7.00 | 5.18 | 0.85 | 4 |

**Table 3.6** Pod morphometry of PM-III and PM-IV (continued)

| No. | PM-III | | | PM-IV | | |
|---|---|---|---|---|---|---|
| | length(cm) | width(cm) | seed/pod | length(cm) | width(cm) | seed/pod |
| 28 | 6.30 | 1.10 | 6.00 | 5.25 | 0.85 | 3 |
| 29 | 5.60 | 0.80 | 5.00 | 3.68 | 1.04 | 1 |
| 30 | 5.50 | 0.80 | 5.00 | 3.88 | 0.85 | 2 |
| 31 | 5.80 | 0.85 | 4.00 | 3.96 | 1.16 | 2 |
| 32 | 5.80 | 1.10 | 7.00 | 3.24 | 0.86 | 2 |
| 33 | 5.50 | 1.00 | 5.00 | 3.45 | 0.81 | 2 |
| 34 | 5.80 | 0.90 | 4.00 | 3.42 | 0.74 | 1 |
| 35 | 5.10 | 0.80 | 4.00 | 3.63 | 0.79 | 2 |
| 36 | 6.40 | 0.60 | 6.00 | 4.07 | 0.63 | 4 |
| 37 | 6.20 | 0.80 | 6.00 | 3.75 | 0.81 | 2 |
| 38 | 5.00 | 0.80 | 5.00 | 6.09 | 0.94 | 5 |
| 39 | 7.20 | 0.90 | 5.00 | 4.87 | 0.8 | 4 |
| 40 | 5.30 | 0.80 | 5.00 | 7.47 | 0.89 | 5 |
| 41 | 5.10 | 0.80 | 5.00 | 4.35 | 1.18 | 3 |
| 42 | 5.90 | 0.90 | 6.00 | 4.29 | 0.96 | 3 |
| 43 | 5.70 | 0.85 | 6.00 | 4.65 | 0.77 | 4 |
| 44 | 5.70 | 0.80 | 5.00 | 5.27 | 0.7 | 3 |
| 45 | 6.00 | 0.90 | 7.00 | 4.46 | 0.93 | 3 |
| 46 | 7.00 | 0.80 | 7.00 | 4.59 | 0.86 | 2 |
| 47 | 5.20 | 1.00 | 6.00 | 3.93 | 0.48 | 5 |
| 48 | 5.50 | 1.00 | 6.00 | 4.48 | 1.17 | 3 |
| 49 | 5.60 | 0.90 | 4.00 | 5.49 | 0.79 | 4 |
| 50 | 5.40 | 1.00 | 5.00 | 3.93 | 0.76 | 3 |
| mean | 6.10 | 0.88 | 5.90 | 4.51 | 0.83 | 3.18 |
| S.E.M. | 0.11 | 0.01 | 0.15 | 0.13 | 0.02 | 0.15 |

## 3.5 Experimental design

When the rats showed a diestrous phase (leucocyte cells) on the forth estrous cycle, they were ovariectomized (OVX) under ether anesthesia. The day of ovariectomy was designed as day 1 of the study period. Rat wer devided in 2 major groups; *P. mirifica* treatment group and control group. For each cultivar of *P. mirifica*, rats were divided in to 2 groups (5 rats per group) as follows;

**Table 3.7** Treatment groups of rats.

| 1.Control groups | 1.1 Negative control group<br>Rats were orally treated with 0.7 ml of distilled water for 14 days. |
|---|---|
| | 1.2 Positive control groups<br>Rats were subcutaneously injected with 17β- estradiol for 14 days. |
| 2. PM groups | 2.1 Rats were orally treated with the powder suspension of *P. mirifica* (PM-III) at the dosage of 100 mg/kg BW/ day in 0.7 ml of distilled water for 14 days. |
| | 2.2 Rats were orally treated with the powder suspension of *P. mirifica* (PM-III) at the dosage of 1,000 mg/kg BW/ day in 0.7 ml of distilled water for 14 days. |
| | 2.3 Rats were orally treated with the powder suspension of *P. mirifica* (PM-IV) at the dosage of 100 mg/kg BW/ day in 0.7 ml of distilled water for 14 days. |
| | 2.4 Rats were orally treated with the powder suspension of *P. mirifica* (PM-IV) at the dosage of 1,000 mg/kg BW/ day in 0.7 ml of distilled water for 14 days. |

The treatment schedule was separated into 3 periods: pre-treatment, treatment and post-treatment (Figure 3.1), in each group as follow;

In the pre-treatment period, the rats were administered with 0.7 ml distilled water for 14 days.

In the treatment period, the rats were fed with 100 and 1,000 mg/kg BW/day in 0.7ml DW of *P. mirifica* suspension for 14 days.

In the post-treatment period, the rats were fed with distilled water for 7 days, they were decapitated on the next day (day 35) under ether anesthesia, and the uteus were dissected and weighed. The uteri collected at the end of the post-treament period were manipulated for histological study.
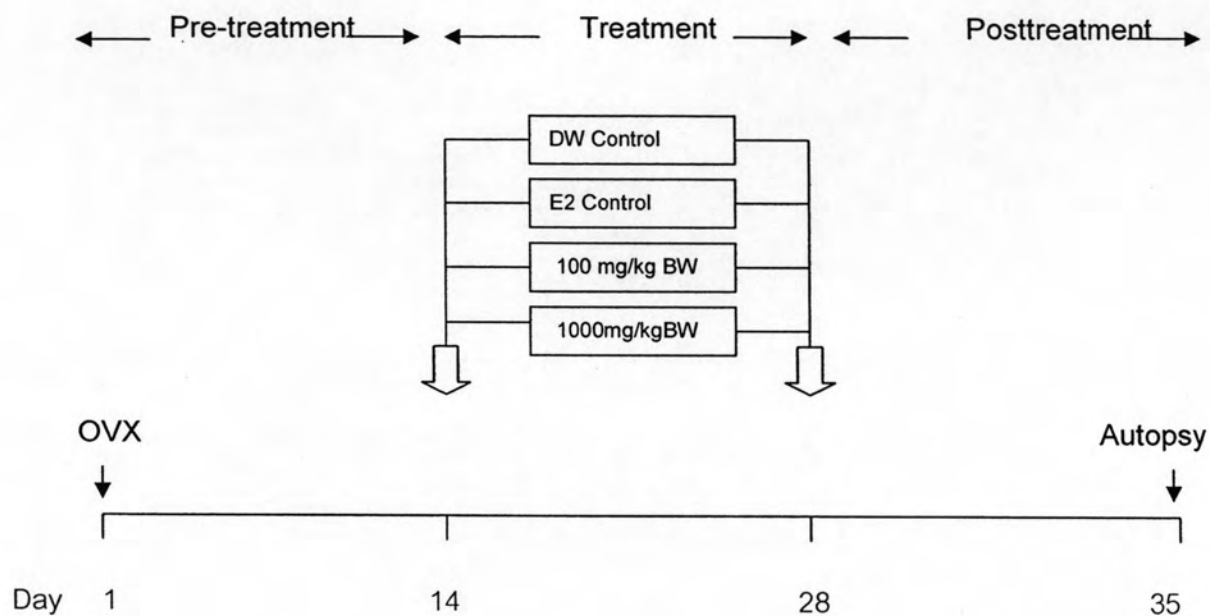


**Figure 3.5**  Diagram of experiment

## 3.6 Vaginal cytology assay

The vaginal smears were checked daily during experiment between 08.00-09.00 hr. A small glass rod was sterilized with 70% alcohol solution and soaked into 0.9% normal saline solution (NSS) before use. The glass rod was inserted into the vagina against the vaginal wall, then smear the vaginal cells into a drop of 0.9% NSS on a slide. The vaginal cells were observed under the light microscope (100X) and recorded the cell type. The cell-type was classified as follows (Figure 3.2);

O       =       the nucleated cells found in the proestrous period.

Co     =       the cornified cells found in the estrous period.

L       =       the leucocyte cells found in both metestrous and diestrous
                periods.
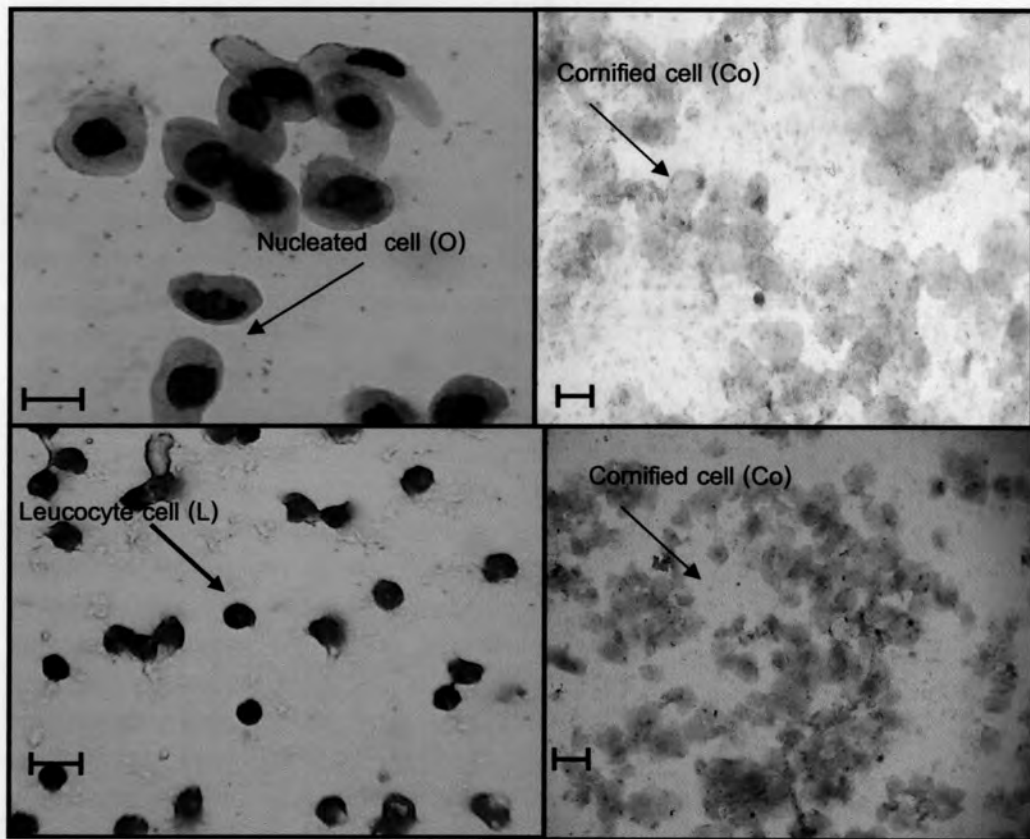


**Figure 3.6** Vaginal epithelium cells found in the different phases of estrous cycle in rats. L-type cells, O-type cells, Co- type cells indicate leucocyte, nucleated and cornified cells, respectively (The scale bar represented 10 μm).

The representative cell-type was chosen from the majority. Vaginal smear cells counts were performed randomly on 100 cells. The percentage of cornified cells was calculated according to Terenius (1971) as follows;

Percentage of cornified cells = $\dfrac{\text{Number of cornified cells}}{\text{Number of leucocytes + cornified cells + nucleated cells}} \times 100$

## 3.7 Uterotrophic assay

The uterus was removed, trimmed the fat tissue, and cut just above the junction between the cervix and the uterine horns. The uterus was weighed and fixed in 10% buffer formalin at least 24 hours and processed according to the standard histological techniques (Humanson, 1979).

## 3.8 Histological study

### 3.8.1 Uterine gland number assay

The number uterine glands were counted in a visual field of x10 magnification, and three uterine tissues from each of five tissues and six randomly chosen fields per tissues were analyzed. Total glands were counted for each uterine section.
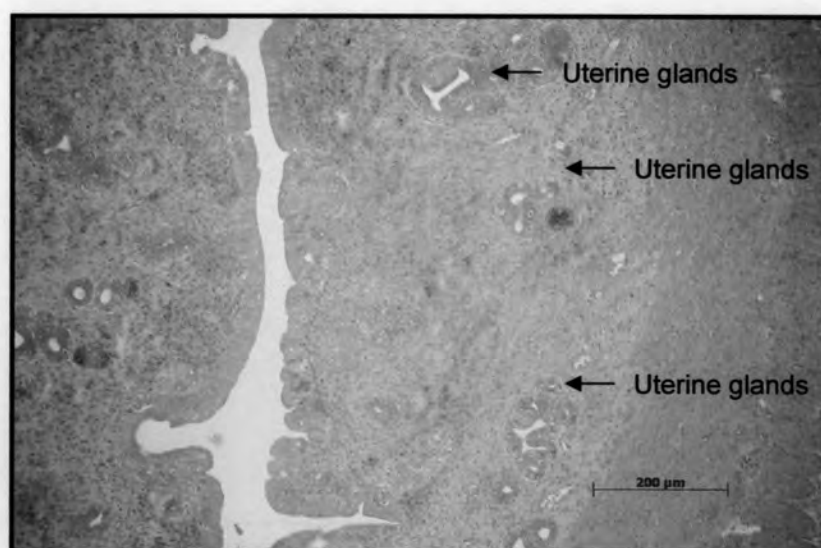


**Figure 3.7** The uterine glands of uterus tissue in ovariectomized rat. Magnitude =x5 (The scale bar represented 200 μm).

### 3.8.2 Cross-section area of uterine tissue assay

The cross-section areas of uterine tissues were measured from a visual field of x5 magnification of the cross-section of uterus by Image-Pro express program version 2. The results were determined as cross-section areas of endometrium, myometrium and lumen of uterus.
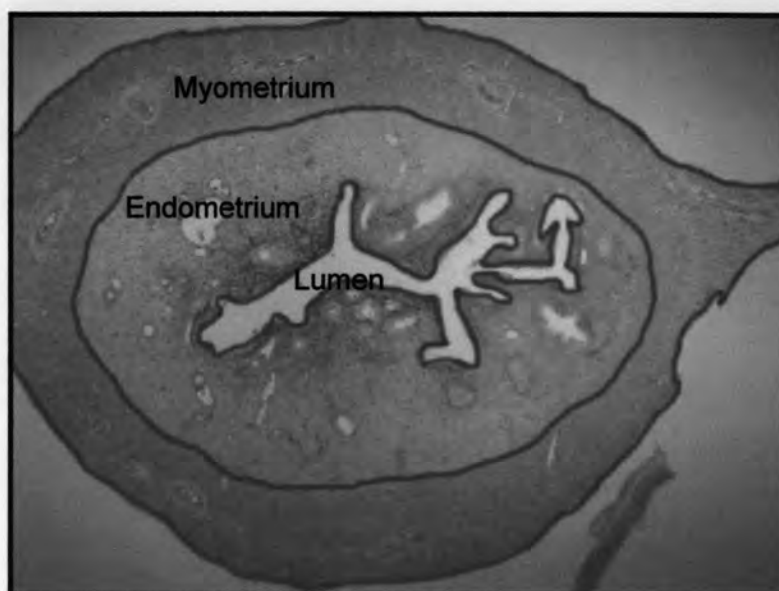


**Figure 3.8** The The area of three layers of uterus tissue in OVX rats. (myometrium, ,endometrium and lumen). Magnitude =x4

### 3.9 Statistical analysis

The results from vaginal cytology were compared between dosage and seasons of collected the sample to seek for the high estrogenic activity that have the earliest and longest response.

Uterine weight, body weight, uterine glands number and cross section area of uterine tissue were analyzed by one way analysis of variance using a PC-based version of the Statistical Program for the Social Sciences (SPSS) program version 11.5.

The correlation between in estrogenic activity determined by vaginal cytology assay and physical factor, or between the estrogenic activity determined by vaginal cytology assay and the isoflavone content are also analyzed by SPSS program, version 11.5. The significant level was taken at $P \leq 0.05$.

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