

Toxicity Study of *Pueraria mirifica* Airy shaw et Suvatabandhu

Songpol Chivapat
Pranee Chavalittumrong
Sadudee Rattanajarasroj
Anchalee Chuthaputti
Somkiat Panyamang

Institute of Medicinal Plant Research
Department of Medical Science

Running titles: Toxicity of *Pueraria mirifica*

of which the ovaries are cut out, we found that the weight and fluid levels in the womb increased in the same manner as was found in mice receiving ethinyl-estradiol. In addition, there is a report that pueraria mirifica worked well in female mice, as a contraceptive at the dosage of 1 gram per week. As for the male mice, pueraria mirifica reduced the sexual activity of mice, by reducing testicular weight, epididymis, adrenal glands, seminal vesicles, sperm counts and the percentage of sperm motility.

In a toxicity study, it was shown that a water extract of pueraria mirifica killed mice with surgically removed ovaries, within 2-3 minutes, but produced estrogenic effects by increasing womb weights, when the dosage was lowered. The study showed that when mice were fed 100 mg per kilogram of body weight, appetites decreased, liver cells became infected and bled, sperm counts lowered and Leydig cells were destroyed. In a study of Japanese partridges, it was found that pueraria mirifica caused swelling and a profusion of pus throughout the body. Additionally, there is a report that pueraria mirifica affected red blood cell production in Japanese partridges by significantly decreasing hematocrits, quantities of hemoglobin and red blood cells.

We can see from the scientific data that pueraria mirifica works similar to estrogen, because it contains many types of phytoestrogens. We acknowledge that before pueraria mirifica could be used safely and effectively, further study on toxicity was necessary. Therefore, we the researchers conducted acute toxicity studies and sub-chronic toxicity studies on pueraria mirifica in animals, to acquire information supporting the future use of pueraria mirifica.

Sub-chronic toxicity test

White rats were divided into five groups, (30 for each group 15 of each sex). The first group, the control group, received distilled water at 5 milliliters / kg / day. The second, third and fourth groups, the test groups, received water with *Pueraria mirifica* (1:5) at 10, 100 and 1000mg / kg / day respectively, given orally, consecutively for ninety days. The fifth group was assigned as the recovery group (1000-R). It was given water with *Pueraria mirifica* at 1000 mg / kg / day for ninety days, then stopped for two weeks, before dissecting and conducting blood tests. This study was directed to find abnormal changes or pathological changes caused by drugs and to see if these changes are reversed in 2 weeks, after discontinuing *pueraria mirifica*.

During the study, researchers observed any abnormalities, changes in physical appearance, recorded weights and amounts of food that rats consumed weekly. After ninety days, the rats fasted for 16 hours and sedated using ether. Researchers dissected, drew blood from posterior vena cava to record the blood values including hematocrit, red blood count, hemoglobin, red blood count index, mean corpuscular volumes (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), number of white blood cells, percent of neutrophil, percent of eosinophil, percent of lymphocytes, percent of monocytes, percent of basophils, numbers of platelets, and percent of reticulocytes by using Automatic Hematological Analyzer (model Cell-Dyn 3500 made by Abbott). Subsequently, blood was serum fractionated to find the values of clinical bio-biochemistry: including levels of alkaline phosphates enzyme (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, BUN, creatinine, glucose, uric acid, triglycerides, cholesterol, sodium, potassium, and chloride ions using Automatic Blood Chemistry Analyzer (Hitachi 912)

After that, researchers dissected the rats to find observable Pathology (gross lesions) of internal organs including brain, heart, lung, windpipe and esophagus, stomach, liver, kidney, spleen, pancreas, colon, bladder, bones (femur), ovaries, testicles, womb, salivary glands, lachrymal glands, mammary glands, endocrine glands including the thyroid and adrenal glands. Researchers noted down weights of organs which could be weighted, to find the percent of relative organ weight and stored those organs in 10% buffered formaline in order to prepare tissue slides and micro-examination and search for changes related to pathology, by Pathologists.

group receiving 1000 mg / kg / day of Pueraria mirifica had a significantly higher percentage of reticulocytes than the control group. The male rats given 1000 mg / kg / day of Pueraria mirifica had significantly lower white blood cell count and lower platelets than the controlled group. All of the male rats groups had significantly lower percentages of basophil than the control group. In the recovery group, it was found that male rats still had significantly lower white blood cell counts and lower platelets than the control group. Female rats groups had significantly lower red blood cell counts and quantities of hemoglobin, than the control group.

Results of Bio-Chemist Test (table 4 & 5)

The group of male rats given 1000 mg / kg / day of Pueraria mirifica had significantly higher levels of ALP then the control group. While ALP values in female rats groups of 10 and 1000 / mg / kg / day were significantly higher. The value of ALT and AST in each male rats group were not different. While ALT values in the female rats receiving 1000 mg / kg / day is significantly higher than the controlled group. The gross protein of the male groups that received 100 and 1000 mg / kg / day was significantly lower then the control group. The levels of albumin, bilirubin, creatinine, and uric acid of all male rats groups were significantly lower than the control group. The male group that received 1000 mg / kg / day had lower levels of triglyceride than the control group while in the female rats group that received the same amount of Pueraria mirifica, had significantly higher levels of triglycerides than the control group. All groups of male and female rats groups receiving 100 and 1000 mg / kg / day of pueraria mirifica had significantly lower cholesterol than the control group. Levels of sodium, potassium and chloride of each male rats group were not different. While the group of female rats that received 1000 mg / kg / day of pueraria mirifica had significantly higher levels of sodium, than the control group.

As for data of biochemistry of the recovery group, two weeks after stopping Pueraria mirifica, male rats had significantly lower levels of albumin, bilirubin, creatinine, uric acid and cholesterol then the control group, while the female group did not show changes seen in the group that received 1000 mg / kg / day.

Table 2,3,4 and 5

The results of internal organ micropathology, (table 10 & 11) show changes as follows:

There were insignificant sporadic infections of cardinal muscles (focal myocarditis) found in male rats of the control group and the test group receiving 10, 1000 mg / kg / day of pueraria mirifica. Accumulation of lymphoid cells around small windpipes and lungs can be found in every group of both sexes of rats and growth rates of occurrence decreased significantly in male rats groups that received 10 and 100 mg / kg / day of Pueraria mirifica ($P < 0.05$). Rate of occurrence of degradation of liver cells (hepatocyte degeneration) of male rats in the control group and the test group were not different while it can be insignificantly seen only in the female group that received 1000 mg / kg / day of Pueraria mirifica. tubular casts around the kidney at the corticomedullary junction, can be somewhat seen in the male rats group receiving 1000 mg / kg / day, but after discontinuing Pueraria mirifica the rate of occurrence of a significant tubular cast increased ($P < 0.05$). The group of female rats receiving 1000 mg / kg / day of pueraria mirifica had a significantly higher rate of occurrence in developing tubular cast than the control group ($P < 0.05$). Focal lymphoids also can be found at small intestine around the ileum, but insignificantly. There were changes in the reproductive system, including significantly higher hyperemia of testicles of the group of male rats that received 1000 / mg / kg / day of pueraria mirifica and some can be found in the group receiving 100 mg / kg / day ($P < 0.05$). It is possible in seminiferous tubules of all groups of rats that received pueraria mirifica, for the development of spermatozoa. In every group of female rats there was no abnormality of ovaries or mammary glands. The wombs of rats that received the highest dosages of pueraria mirifica had subendometrial gland hyperplasia, but the rate was insignificant. After discontinuing pueraria mirifica, the rate of occurrence increased significantly ($P < 0.05$). The occurrence of fatty degeneration at the cortex layer of adrenal gland can be found in the control group of male rats and similarly to groups receiving 10 and 100 mg / kg / day of Pueraria mirifica. There was no abnormality detected at the thyroid gland, lachrymal glands, salivary and bones of all groups of both sexes.

Table 10 and 11

the hematocrit values and reticulocyte returning to normal. Even if red blood cell volume and hemoglobin were significantly lower than the control group, there was a trend toward increase. The decrease of MCHC values at 10g./ kg./ day in female rats groups should not only be from pueraria mirifica, as the results were neither increasing or decreasing in relation to the dose of pueraria mirifica. The amount of white blood cells and platelets in male rats group with 1000 mgs./ kg./ day were significantly less than the control group after having stopped the dose for 2 weeks were still significantly lower than the control group. It may be the result of pueraria mirifica having some phytoestrogens that have an effect in decreasing or stopping the formation of blood cells in bone marrow , in the same way that estrogen depresses bone marrow and causes pancytopenis (Van and Friedland 1987; Bernard; et. Al. 1983) which lowers the formation of red blood cells, white blood cells, and platelets However, in female rats receiving pueraria mirifica in the same doses, did not find any decreases in white blood cells or platelets. It may be caused from gender-related differences. The decrease of basophils in male rats receiving pueraria mirifica was not related to the dose of pueraria mirifica and was not decreased in female rats. It can not be attributed to pueraria mirifica.

The changes of some biochemistry including ALP levels in both genders of rats that received pueraria mirifica at 1000 mg./ kg / day was significantly increased compared to the control group, but still within the normal range 56.8-128 U/L (Shayne, 1992). Bilirubin values, in the micro pathology results of the mouse liver, did not show any blockage in the bile duct, so changes were not specific to a toxicity of pueraria mirifica. The statistical increase of ALT enzyme levels in the female mouse group taking 1000 mg./ kg / day, is important but not significant, to show the presence of hepatic injury. If a hepatic injury occurs, ALT enzyme will increase obviously. The protein in male rats that received 100 and 1,000 mgs / kg / day of pueraria mirifica is statistically less than the control group, but is still within the normal range. The albumin decreased in male rats. After pueraria mirifica was stopped the albumin was still significantly lower than the control group. It shows that pueraria mirifica may interrupt the albumin reproduction in the liver. However, the resulting values are still within in normal range (Shayne, 1992). There was no decrease in serum protein and albumin in female rats. The cause may be gender-related differences. The uric acid in every group of rats in the experiment is statistically decreased but still within the normal range and did not show any sign of abnormality. The triglycerides in female rats receiving pueraria mirifica increased relative to the pueraria mirifica dosage, especially in the group that received pueraria mirifica in 1,000 mg / kg / day This demonstrates that pueraria mirifica may cause

production of FSH hormone that is necessary to the development of seminiferous epithelium. It was found that pueraria mirifica at 100 and 1,000 mgs / kg / day in female rats effects uterine distention and has greater fluid content and more weight which is similar to the report of Smitasiri et. Al. (1986) that found, pueraria mirifica powder effects the mouse womb even with cut fallopian tubes and ovaries removed and has more weight and more fluid content. The effect of pueraria mirifica powder at 1-mg dosage is as strong as ethnylestradiol at 0.52 - 0.75 micrograms.

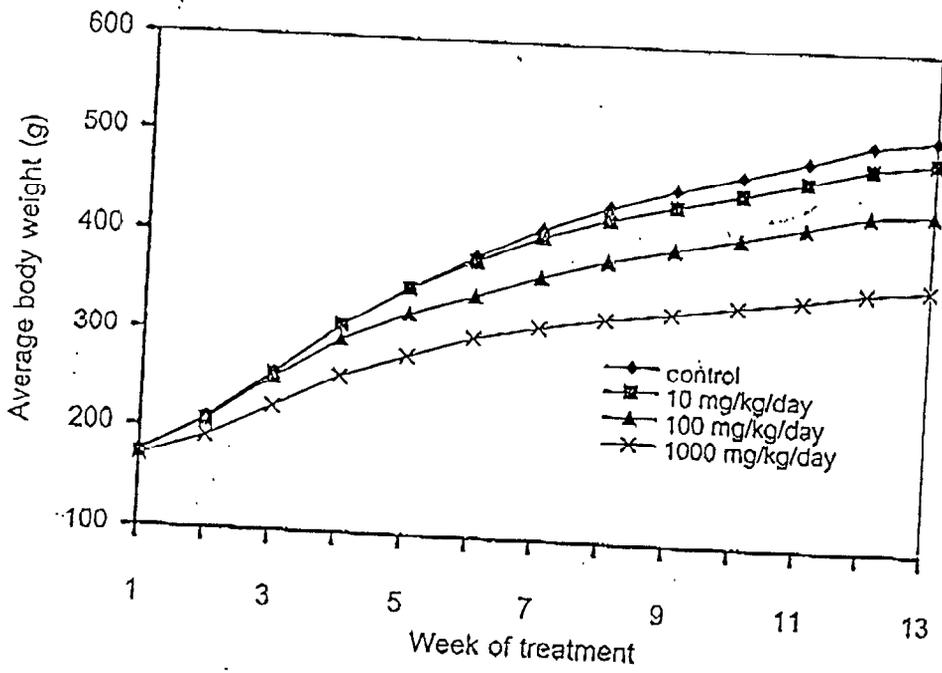
The micro-pathological changes in organs is peribronchiolar lymphoid aggregation in male rats lungs that receive pueraria mirifica in 10 and 100 mgs / kg / day. There is a lower rate of occurrence than the control group but it was not related to the dose of pueraria mirifica and in female rats the rate was not different. So it can be concluded that this change does not come from pueraria mirifica. The degeneration in liver cells in both control and experiment male rats were no different and found to be insignificant in the female rats group that received 1,000 mgs / kg / day, so it can concluded that this change does not come from pueraria mirifica. Tubular cast in male mouse kidneys that received pueraria mirifica at 1,000 mgs / kg / day has a very low growth rate, so the rate of tubular cast increasing after having stopped for 2 weeks, should not come from pueraria mirifica. However, in female rats, pueraria mirifica at 1,000 mgs. kg / day makes tubular casts grow at an increased rate. However this study has found casts in kidney tubes, but has not found any injury to the kidney cells and cannot report that pueraria mirifica is toxic to the kidney. The hyperemia in testicle tissue of male rats receiving pueraria mirifica at 1,000 mgs / kg / day increased to a mild degree but not to a dangerous degree. The subendometrial hyperplasia gland in the womb of rats receiving pueraria mirifica at 1,000 mg / kg / day increased insignificantly, but after stopping the dose for 2 weeks the rate is significantly increased. The cause maybe pueraria mirifica has an estrogenic effect, affecting the production of gonadotropin. An experiment found that 83% of rats that receiving coumestrol which is a type of phytoestrogen found in pueraria mirifica has a persistent estrous state and when given estrogen type estradiol, it can not increase LH hormone in these rats. This result shows coumestrol effects the growth of neuroendocrine impairment (Whitten, et. Al 1993). In the same way, giving a high dose of pueraria mirifica may cause the FSH hormone to stop rats and causes the estrogen from ovaries to decrease and may cause low progesterone. These 2 hormones help the growth in membranes and glandular materials of the uterine wall, so we can not find endometrial gland hyperplasia in most rats, only a few can be detected. After stopping pueraria mirifica, some rats may have menses return back to normal, so

Conclusion

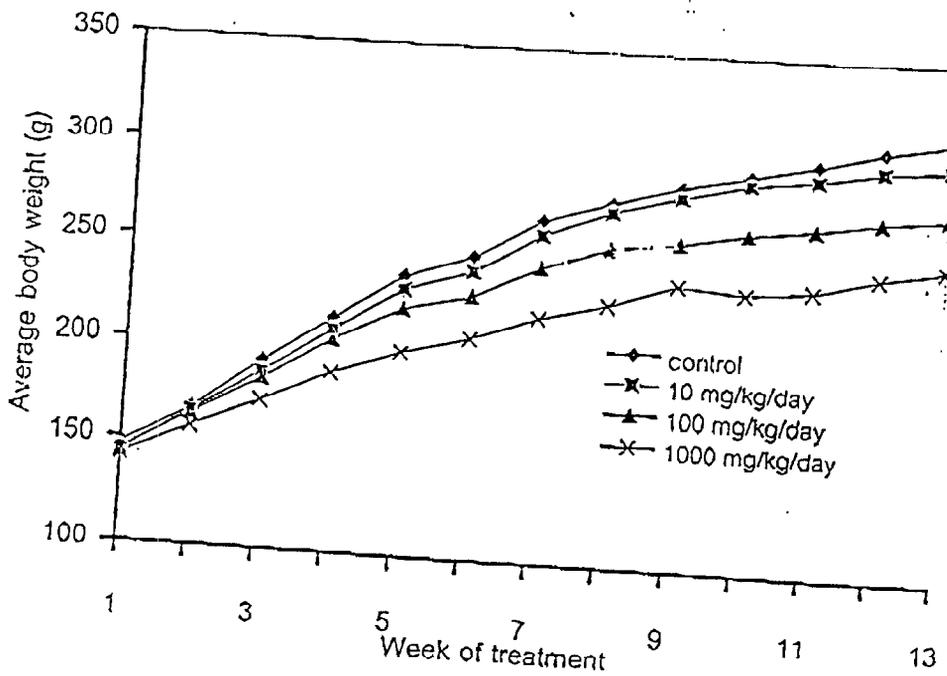
From the acute toxicity study of hamsters, we found that pueraria mirifica did not cause any acute toxicity that can kill half of the hamsters and the LD-50 value is higher than 16 g per kg of the hamster's body weight. From the sub-chronic study that gives 10, 100 and 1000 mg / kg / day of pueraria mirifica in liquid form to rats for 90 days, we found that rats in the group that obtained 100 and 1000 pueraria mirifica had a slower growth rate and less appetite than the control group. Pueraria mirifica at 1000 mg / kg / day affects the blood of both rats groups. Percentage of hematocrit, red blood cell count, and quantity of hemoglobin were significantly lower than in the control group and the percent of reticulocyte was more than the control group. Moreover, white blood cells and plasma in male rats was reduced significantly. Researchers also found that pueraria mirifica at 10, 100 and 1000 mg / kg / day significantly reduced cholesterol in male rats, while cholesterol in female rats was reduced only in the group that received pueraria mirifica at 100 and 1000 mg / kg / day. Some values from the biochemical studies only changed within normal ranges. Regarding the study of pathology of internal organs, researchers found that pueraria mirifica at 1000 mg / kg / day can significantly bring about a cast in the kidney tubes of female rats significantly more often than in the control group, but it is not the case in male rats. Pueraria mirifica at 1000 mg / kg / day reduces weights of male rats testicles and had little hyperemia in related tissue. Pueraria mirifica at 100 and 1000 mg / kg / day swells the wombs of female rats due to high fluid content and makes them heavier. Changes of other organs are not related the dosage of pueraria mirifica; therefore, are not caused by Pueraria mirifica. This toxicity study shows that receiving pueraria mirifica for a long time can affect creation of blood cells in white rats. Consequently, if people want to take pueraria mirifica as pills, it is not recommended for a long periods of time, as it may affect the production of blood cells.

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Picture 1 Average body weight of male rats, receiving Pueraria Mirifica for 90 days.



Picture 2 Average body weight of female rats, receiving Pueraria Mirifica for 90 days.

Table 1

Average increase of body weight and consumption of rats, receiving *Pueraria mirifica* Mirifica for 90 days.

Dose of <i>Pueraria mirifica</i> (mg/kg BW/day)	n/sex	Body weight gain (g)	Food daily intake (g/rat/day)
0	15/M	338.27 ± 30.40	22.99 ± 1.86
10	15/M	317.27 ± 36.74	21.79 ± 1.73
100	14/M	264.50 ± 37.94*	20.25 ± 1.18*
1000	15/M	194.33 ± 25.05*	17.96 ± 1.51*
0	15/F	160.67 ± 16.88	17.23 ± 1.18
10	15/F	156.53 ± 24.73	15.78 ± 1.14
100	14/F	127.71 ± 9.63*	15.24 ± 1.24*
1000	15/F	102.40 ± 17.32*	13.62 ± 1.14*

The values are expressed as mean ± SD.

*significantly different from control group (P<0.05)

Table 3

Blood test of female rats receiving *Pueraria mirifica* for 90 days.

Parameters	Dose of <i>Pueraria mirifica</i> (mg/kg BW/day)				
	control	10	100	1000	1000-R
	n=15	n=14	n=14	n=15	n=15
Hematocrit (%)	51.24 ± 5.88	53.04 ± 1.92	50.86 ± 2.84	45.90 ± 1.76*	50.67 ± 1.92
RBC (× 10 ⁶ cells/mm ³)	9.12 ± 0.62	9.24 ± 0.29	8.83 ± 0.56	7.94 ± 0.38*	8.69 ± 0.33*
Hb (g/dl)	15.96 ± 0.70	15.64 ± 0.43	15.26 ± 0.78*	13.78 ± 0.49*	14.92 ± 0.41*
MCV (μm ³)	57.54 ± 1.47	57.42 ± 1.40	57.69 ± 2.20	57.87 ± 1.20	58.40 ± 1.83
MCH (pg)	17.58 ± 1.08	16.94 ± 0.63*	17.31 ± 0.66	17.38 ± 0.43	17.20 ± 0.64
MCHC (%)	30.61 ± 2.16	29.52 ± 1.12*	30.02 ± 0.98	30.03 ± 0.53	29.45 ± 0.72*
WBC (× 10 ³ cells/mm ³)	3.68 ± 1.29	4.17 ± 0.98	3.19 ± 0.87	3.66 ± 0.87	3.38 ± 0.91
Neutrophil (%)	14.11 ± 6.20	12.64 ± 6.72	14.99 ± 15.31	12.44 ± 5.35	12.05 ± 3.73
Eosinophil (%)	1.40 ± 0.63	1.60 ± 1.04	1.26 ± 0.55	1.11 ± 0.39	1.21 ± 0.36
Lymphocyte (%)	78.23 ± 6.75	81.61 ± 7.48	80.74 ± 8.57	81.89 ± 6.12	83.02 ± 4.03
Monocyte (%)	4.48 ± 2.12	2.91 ± 2.11	4.89 ± 3.82	3.15 ± 2.09	2.18 ± 1.35*
Basophil (%)	1.76 ± 0.80	1.24 ± 0.80	1.69 ± 0.91	1.42 ± 0.69	1.54 ± 0.58
Platelet (× 10 ³ cells/mm ³)	889.47 ± 113.23	855.46 ± 59.13	907.61 ± 69.69	904.43 ± 89.54	848.47 ± 67.90
Reticulocyte (%)	2.62 ± 0.80	3.08 ± 0.62	2.89 ± 0.69	5.18 ± 1.61*	2.94 ± 0.37

The values are expressed as mean ± SD.

* significantly different from control group

1000-R = Recovery group

Table 5

Data on clinical Bio-Chemistry of female rats
receiving *Pueraria mirifica* for 90 days

Parameters	Dose of <i>Pueraria mirifica</i> (mg/kg BW/day)				
	control	10	100	1000	1000-R
	n=15	n=15	n=14	n=15	N=15
ALP(U/L)	30.73±4.42	36.67±6.08*	30.79±6.47	39.00±8.59*	33.07±4.01
ALT(U/L)	32.33±4.03	30.13±5.10	36.64±4.75	38.73±9.71*	30.13±4.34
AST(U/L)	69.33±10.41	73.47±10.62	63.86±5.57	63.33±10.85	60.00±5.73*
Total protein (g%)	6.90±0.34	6.84±0.43	6.77±0.38	7.18±0.37	6.99±0.35
Albumin (g%)	3.58±0.21	3.62±0.26	3.56±0.25	3.65±0.20	3.59±0.20
Globulin (g%)	3.32±0.18	3.22±0.20	3.21±0.19	3.53±0.25*	3.40±0.18
Bilirubin (mg/dl)	0.09±0.04	0.10±0.03	0.08±0.02	0.08±0.03	0.08±0.04
BUN (mg%)	21.07±4.26	19.89±2.67	19.32±3.65	21.73±5.93	23.49±4.43
Creatinine (mg%)	0.64±0.03	0.64±0.04	0.60±0.05	0.60±0.05	0.61±0.06
Glucose (mg/dl)	149.56±22.15	143.83±25.97	151.83±27.14	156.39±16.91	144.52±20.64
Uric acid (mg/dl)	1.99±0.87	1.67±0.57	2.04±0.95	1.84±0.47	1.59±0.48
Triglyceride(mg/dl)	99.34±44.66	104.60±73.26	121.27±40.08	151.21±86.66*	129.48±55.96
Cholesterol (mg/dl)	71.82±12.19	64.96±10.04	54.78±13.24*	28.30±10.72*	79.14±13.50
Na ⁺ (mmol/l)	142.47±1.19	142.93±1.44	142.86±1.46	144.60±2.53*	142.07±2.79
K ⁺ (mmol/l)	5.69±1.01	5.41±0.94	5.61±0.82	5.41±0.55	5.33±0.72
Cl ⁻ (mmol/l)	110.47±3.27	111.40±2.80	111.93±3.25	112.00±2.80	110.67±3.81*

The values are expressed as mean ± SD.

* significantly different from control group

1000-R =Recovery group

Table 7

Organ weight of female rats receiving *Pueraria mirifica* for 90 days.

Organs	Dose of <i>Pueraria mirifica</i> (mg/kg BW/day)				
	control	10	100	1000	1000-R
	n=15	n=15	n=14	n=15	n=15
Brain	1.918±0.086	1.898±0.055	1.910±0.092	1.868±0.057	1.884±0.076
Heart	0.902±0.079	0.884±0.071	0.865±0.082	0.792±0.085*	0.828±0.073*
Lung	1.291±0.083	1.207±0.082	1.224±0.111	1.137±0.111	1.166±0.113
Stomach	1.552±0.279	1.430±0.190	1.537±0.191	1.445±0.154	1.491±0.199
Liver	7.307±0.550	7.049±0.962	7.225±0.546	7.519±0.885	7.257±0.869
Right Kidney	0.844±0.065	0.822±0.087	0.821±0.062	0.799±0.105	0.832±0.067
Left Kidney	0.780±0.057	0.785±0.081	0.779±0.056	0.773±0.107	0.780±0.064
Spleen	0.711±0.109	0.726±0.083	0.697±0.085	0.649±0.098	0.642±0.080
Uterus	0.764±0.107	0.852±0.142	0.904±0.180*	0.898±0.187*	0.826±0.182
Bladder	0.084±0.014	0.086±0.013	0.081±0.010	0.085±0.012	0.078±0.014

The values are expressed as mean ± SD.

1000-R: Recovery group

* significantly different from control group

Table 9 Relative organ weight of female rats receiving Pueraria mirifica for 90 days.

Organs	Dose of <i>Pueraria mirifica</i> (mg/kg BW/day)				
	control	10	100	1000	1000-R
	n=15	n=15	n=14	n=15	n=15
Brain	0.641±0.052	0.652±0.061	0.718±0.043	0.790±0.060*	0.759±0.048*
Heart	0.302±0.04	0.303±0.028	0.325±0.032	0.333±0.024*	0.334±0.31*
Lung	0.432±0.039	0.414±0.034	0.460±0.038	0.479±0.035*	0.469±0.048*
Stomach	0.521±0.114	0.491±0.074	0.579±0.082	0.609±0.057*	0.602±0.093*
Liver	2.436±0.160	2.404±0.245	2.715±0.209*	3.166±0.321*	2.913±0.263*
Right Kidney	0.282±0.023	0.281±0.028	0.309±0.024*	0.336±0.039*	0.334±0.018*
Left Kidney	0.260±0.017	0.268±0.022	0.293±0.023*	0.325±0.033*	0.314±0.021*
Spleen	0.237±0.033	0.249±0.036	0.262±0.029	0.272±0.029*	0.258±0.030
Uterus	0.255±0.039	0.294±0.061	0.340±0.069*	0.376±0.064*	0.332±0.071*
Bladder	0.028±0.005	0.029±0.006	0.031±0.004	0.036±0.004*	0.032±0.005

The values are expressed as mean ± SD.

1000-R = Recovery group

* significantly different from control group

Table 11

The result of microscopic pathology examination of female rats receiving *Pueraria mirifica* for 90 days.

Organs	Microscopic findings	Dose of <i>Pueraria mirifica</i> (mg/kg BW/day)				
		control n=15	10 n=15	100 n=14	1000 n=15	1000-R n=15
Brain		NR	NR	NR	NR	NR
Lung	Peribronchiolar lymphoid aggregation	6/15	7/15	10/14	8/15	10/15
Liver	Hepatocyte degeneration	0/15	0/15	0/14	2/15	0/15
Kidney	Tubular cast	2/15	0/15	2/14	8/15*	6/15
	Lymphoid aggregation	1/15	2/15	0/14	0/15	0/15
Spleen		NR	NR	NR	NR	NR
Intestine	Local lymphoid hyperplasia	1/15	0/15	0/14	0/15	0/15
Pancrease		NR	NR	NR	NR	NR
Bladder		NR	NR	NR	NR	NR
Ovary		NR	NR	NR	NR	NR
Uterus	Subendometrial gland hyperplasia	0/15	0/15	0/14	2/15	5/15*
Mammary glands		NR	NR	NR	NR	NR
Thyroid glands		NR	NR	NR	NR	NR
Adrenal glands		NR	NR	NR	NR	NR
Bone		NR	NR	NR	NR	NR

The results are expressed as number of rats with pathological findings/ total number of rats examined.

*significantly different from control group ($P < 0.05$)

NR = No remarkable lesion