Studies on the Toxicity of Pueraria mirifica in Tripala-treated Female Rats

Yuthana Smitasiri
Nirush Lertprasertsuk
Athipong Manasathien
Sitthisak Pinmongkhongul
Alex Schauss

Mae Fah Luang University
Amphor Muang, Chiang-rai Province

Introduction

It is known that White Kwaw Krua (Pueraria mirifica A Shaw. & Suvat.) possesses estrogenic effects. It was found that the tuberous root of Pueraria mirifica contains phytoestrogens such as Coumestrol, Diadzein, Daidzin, Genistin, Genistein, Miricifin, Puerarin, and Miroestrol. These constituents are sources of the estrogenic-like effects that Pueraria mirifica exhibits.

In the drug pamphlet of Luang Anusarn Sunthorn on the application of Pueraria mirifica (1931), Pueraria mirifica is a miracle herb that has rejuvenating properties and promotes good health. However, it was indicated, in the pamphlet, that Pueraria mirifica should be taken with other herbs such as Tripala, for safety and efficacy.

Tripala is a Thai traditional herbal medicine, comprising Terminalia bellerica, Terminalia chebula, and Phyllanthus emblica. It was reported that Tripala might be used to adjust a patient's element during the summer. It is composed of dried fruits of Terminalia bellerica, Terminalia chebula, and Phyllanthus emblica in different ratios, based on the traditional diagnosis of the patient.

Other names of Terminalia bellerica are Chibadu (Karieng* in Chiangmai), Lun (Chiangrai), Samor Nae (the central region), Saku (Karieng in Mae Hong Sorn), Nae, Nae Khao, Nae Ton (Northern region). The scientific name is Terminalia bellerica Roxb. It belongs to Combretaceae family. The traditional medicinal applications are to use the fresh fruit as a laxative and the ripe fruit for wound healing, abdominal dropsy and hemorrhoids.

In Myanmar, the dried fruit is used to alleviate coughing and eye problems. In Indochina, Terminalia bellerica is used to heal incisions and as a tonic, while the fresh fruit is used as a laxative. From the chemistry, it was found that Terminalia bellerica consists of β-sitosterol, gallic acid, ellagic acid, ethyl gallate, galloyl glucose, chebulagic acid, mannitol, glucose, galactose, fructose, rhamnose, cardiac glycoside and belericoside. Pharmacological study shows that the β-sitosterol, found in Tripala, may reduce cholesterol absorption and decrease the concentration of cholesterol in the blood. It is used in pharmaceuticals as a hypolipidemic agent, for persons experiencing prostate gland problems. Gallic acid was used as astringent, as a styptic and as an intestinal astringent in animal medications. Ellagic acid was used as astringent, and as hemostatic for external application. In addition, the extract of Terminalia bellerica in ethanol may increase bile flow in dogs and reduce blood pressure to a level that can cause death. The LD_{50} in rats is 4.25 g/kg by oral administration. However, the water extract of Terminalia bellerica is not very effective in increasing bile flow and insignificantly reducing blood pressure. Rats dosed with 5 g/kg of Terminalia bellerica, experienced no toxic symptoms.

Terminalia chebula have many others names, Manae (Karieng in Chiangmai), Samor up yah (central region), Mak nae (Karieng in Maehongsorn). The

scientific name is Terminalia chebula Retz, belonging to Combretaceae family. Traditional medicinal application indicates that fruits are used for expelling phlegm. Anti dysentery, relief of fever, relief of symptoms due to bile and for insomnia.

Chemical study shows that the fruit of Terminalia chebula consists of β-sitosterol, gallic acid, ethyl gallate, galloyl glucose, chebulagic acid, mannitol, glucose, galactose, fructose, rhamnose, chebulenic acid, tannic acid, terchibin, brevifolin, and chebulic acid. Pharmacological study shows that Terminalia chebula extract may inhibit Samonella and Shigella bacteria. Moreover, the study of toxicity of Terminalia chebula in rats shows that it caused wounds in the liver and kidneys and also caused abnormalities in the vena cava.

The scientific name of the last constituent of Tripala is Phyllanthus emblica Linn. (Emblica officinale Gaertn.), belonging to Euphorbiaceae family. The traditional medical application is to use the dried fruit for healing sore throats and coughs. Juice from the fresh fruit is a diuretic and can alleviate diarrhea. The juice can also be used as eye drops for eye infections.

Chemical study shows that its fruit consists of ascorbic acid, trigalloylglucose, terchebin, corilagin, ellagic acid, 3,6-digalloylglucose, glucogallin, gallic acid, and amino acid (glutamic acid, proline, aspartic acid, alanine, lysine, cystine). The pharmacological study shows that chebulagic acid had cytotoxic affects on melanoma cells and inhibited the topoisomerase I enzyme, which is necessary for cell replication. In addition, the water extract of Phyllanthus emblica, using Shibata et al, and Taesotikul and Kanjanapothi method, may reduce blood pressure in mice. The acute toxicity study in mice, treating mice with 0.1 and 0.5 grams of Phyllanthus emblica water extract per 1 kilogram of body weight for 10 weeks, showed that there was no effect in the growth of mice, but affected the heart, lung and liver weights and increased SGPT serum levels. When giving 20 g/kg of extract to mice by oral administration, there was no toxicity found and when the extract was injected through the abdomen of the mice, the LD_{50} was at 0.415 g/kg in male mice and 0.288 g/kg in female mice.

Moreover, it was reported, Tripala is potent for cholesterol reduction in rabbits receiving cholesterol with Terminalia bellerica, cholesterol with Terminalia chebula, cholesterol with Phyllanthus emblica and only cholesterol. The result was that cholesterol levels in the serum of rabbits receiving cholesterol with Terminalia bellerica, Terminalia chebula, and Phyllanthus emblica were significantly lower than those receiving only cholesterol.

The fact that there have been many doses of Pueraria mirifica, ranging from very low to very high suggested to consumers by retailers, the Department of Medical Science of the Ministry of Public Health, Thailand has set limits for the daily dosage not to exceed 100 mg. and announced that it should be taken along with complementary herbs, such as Tripala.

This study, therefore, aims to examine the acute toxicity, sub-acute toxicity, sub-chronic toxicity, and chronic toxicity, whether the suggested usage of Pueraria mirifica with Tripala by Luang Anusarn Sunthorn is not toxic and is safe for the consumer.

Materials and Methods

Test animals

Wistar rats from the National Laboratory Animal Center, Mahidol University were sent to the Mae Fah Luang University by air. After the arrival, rats were rested and reared in an air-conditioned room at 24°±1° C. The light was controlled for 12 hours light and 12 hours dark. Feed used in the study is Mice Feed No. 082 from Charoenpokaphand Food Public Company Limited. Food and water were provided sufficient for 24 hours. The bottom of the rat cage was laid with sterilized sawdust from the National Laboratory Animal Center.

Herbs

1) Pueraria mirifica - A Shaw. & Suvat.

Pueraria mirifica was obtained from Amphor Mae Loa, Chiang-rai province. The weight of the tuberous root were between 1.126 ± 0.108 kg. The tuberous roots were washed with water, peeled, sliced and dried in the oven at 60° C until they were completely dry. The dried slices of Pueraria mirifica were then ground and stored in sealed compartments.

2) Tripala

Fresh crude of Terminalia bellerica, Terminalia chebula, and Phyllanthus emblica from sources in Chiang-mai province were used in this study. Fresh crude was dried in oven at 60° C until they were completely dry. Tripala used in this study is a composition of Terminalia bellerica, Terminalia chebula, and Phyllanthus emblica at 1:1:1 ratio, well blended together and then stored in sealed compartments, ready for the study.

The toxicity study (1,3,6 and 9, months) of Tripala (based on human doses) mixed with Pueraria mirifica at different doses (human dose, 10 fold of human dose and 100 fold of human dose) in female rats

The dosage of Tripala and Pueraria mirifica was from mixing Terminalia bellerica, Terminalia chebula, and Phyllanthus emblica at human dose of 1:1:1 ratio (150 mg. in total) and mixed it with Pueraria mirifica at 100mg. (the recommended dose, for a 50 kg. person/day). The study maintained the dose of Tripala (at 150 mg.), but increased the dose of Pueraria mirifica to 10, 100 fold of the human dose.

Grouping of the test rats

The virgin female Wistar rats from the National Laboratory Animal Center, Mahidol University, weighing between180-200 grams, were sent to Mae Fah Luang University and reared in the animal laboratory animal house as previously mentioned.

Test rats were divided into 5 groups, eight rats in each group

Group 1 was the control group receiving distilled water at 1 ml./rat/day by gavage in the morning.
Group 2 received Tripala mixed with water at 0.6 mg./rat/day by gavage in the morning (equivalent to the human dose)
Group 3-5 received Tripala mixed with Pueraria mirifica by gavage. The dose of Tripala is 0.6 mg./rat/day while the dose of Pueraria mirifica was 0.4, 4 and 40 mg./rat/day, respectively. The dose of Pueraria mirifica was equivalent to the human dose (100 mg./day), 10 fold the human dose and 100 fold the human dose.

Procedures of acute, sub-acute, sub-chronic, and chronic toxicity study of Tripala mixed with Pueraria mirifica.

1) Acute, Sub-acute Toxicity

The substance (Tripala + Pueraria mirifica) was given to rats for one day. The test rats were observed every 30 minutes for 6 hours.

In the event that no sign of abnormality was seen, the substance would continue to be given to rats everyday for 4 weeks(subacute toxicity test). The rats would be weighed every week and any noticeable change would be recorded. After the last weighing of the rats, they were sacrificed and the liver, kidney, heart, spleen, ovary, uterus, adrenal glands, brain, and pituitary gland, are grossly examined and then weighed by the 4-digit scale electronic balance (Metler: Model Ab 204). All data would be analyzed using statistic methods by using Independent student T-test and Analysis of Variance, least significant difference.

2) Sub-chronic Toxicity (12 weeks)

The substance was given to five groups of rats, 16 rats in each group, for 12 weeks. The rats were weighed every week to record any noticeable changes. After 12 weeks, all the rats would be anesthetized to remove the liver, kidney, heart, spleen, ovary, uterus, adrenal glands, brain, and pituitary gland for weighing by the 4-digit scale electronic balance (Metler: Model Ab 204). All data was analyzed by statistical methods (Independent student T-test; Analysis of Variance, least significant difference). The organs removed from the rats were kept in buffered formalin, chopped, stained with haematoxylin, eosin, to read pathological data using a microscope.

3) Chronic Toxicity (24 weeks)

The substance was given to five groups of rats, 16 rats in each group, for 24 weeks. This study follows the same process of the sub-chronic toxicity (12 weeks), as previously described.

4) Long–period Chronic Toxicity (36 weeks)

The substance was given to five groups of rats, 7-10 rats in each group, for 36 weeks. This study follows the process of the sub-chronic toxicity (12 weeks), as previously described.

Toxicity Report

After the rats were sacrificed at time schedule (12,24, and 36 weeks) and after gross examination of organs removed and weighed then. The organ of interest were liver, spleen, adrenal and pituitary glands, which were involved in the metabolism, body defence and hormonal control. The organs were serially cut into several pieces whereas the others were taken in toto and fixed in 10% neutral-buffered formalin before routinely processed. They were embedded in paraffin as tissue blocks and cut at 4 microns. The tissue sections were stained routinely with Hematoxylin, and eosin for microscopic examination.

Results

1) Acute Toxicity

From the study, it was found that Tripala and Tripala mixed with Pueraria mirifica did not show any acute toxicity in the test rats in the test rats when observed during 6 hours after gavage. (Table1)

2) Sub-acute Toxicity (4 weeks)

The study showed that rats receiving Tripala at 0.6 mg./rat/day for four weeks did not significantly differ between the body weight and the weights of liver, kidneys, heart, spleen, ovaries, uterus, adrenal glands, brain, and pituitary gland, from the control group. (Table 2)

Table 3 shows that Pueraria mirifica, at the three doses, when mixed with Tripala did not alter the body weight and the weight of liver, kidneys, heart, spleen, ovaries, uterus, adrenal glands, brain, and pituitary gland, significantly, from the group receiving only Tripala.

3) Sub-Chronic Toxicity (12 weeks)

The study showed that receiving Tripala at 0.6 mg./rat/day for 12 weeks did not alter the body weight and the weight of liver, kidneys, heart, spleen, ovaries, uterus, adrenal glands, brain, and pituitary gland significantly different from the control group (receiving only distilled water). (Table 4)

Table 5 showed that rats receiving Tripala at 0.6 mg./rat/day with Pueraria mirifica at 0.4 mg./rat/day for 12 weeks had significantly higher kidney weights than the group receiving only Tripala statistically significant at $P<0.05$. It was also found that rats receiving Tripala at 0.6 mg./rat/day with Pueraria mirifica at 40 mg./rat/day for 12 weeks had significantly higher weights of liver, kidney, heart, and brain, than the group that received only Tripala statistically significant at $P<0.01$. However, receiving Tripala with

Pueraria mirifica at the dose of 0.4 or 4 mg/rat/day, did not make the weight of liver, kidney, heart, spleen, ovaries, uterus, adrenal glands, brain, and pituitary gland, significantly different from the group receiving only Tripala statistically significant, except in some groups that were previously mentioned earlier.

4) Chronic Toxicity (24 weeks)

From the comparison of rats receiving only Tripala and those receiving only distilled water, every day for 24 weeks, it was found that Tripala did not cause body weight and the weights of the kidney, heart, spleen, ovaries, uterus, adrenal glands, brain and pituitary gland, significantly different from the control group, except that it caused the liver weight to be significantly less than in the control group ($P<0.05$) (Table 6)

Table 7 shows rats receiving Tripala with Pueraria mirifica at three different doses, everyday for 24 weeks, it was found that the body weight and the weights of liver, kidney, heart, spleen, ovaries, uterus, adrenal glands, brain and pituitary gland, were not significantly different from the rats receiving only Tripala except the liver weight of Pueraria mirifica 40mg/rat/day is significantly more than the Tripala treated group ($P<0.05$)

5) Long-period Chronic Toxicity (36 weeks)

From the study, it was found that giving Tripala to rats everyday for 36 weeks, did not alter the body weight and the weights of the kidney, heart, spleen, ovaries, uterus, adrenal glands, brain and pituitary gland, significantly different from the control group receiving only distilled water (Table 8)

When giving Tripala with Pueraria mirifica at three different doses to rats everyday for 36 weeks, it was found that Tripala mixed with Pueraria mirifica at 3 different doses, did not alter the body weight or the weights of the kidney, heart, spleen, ovaries, uterus, adrenal glands, brain and pituitary gland significantly different from the control group (Table 9), except for the group receiving Tripala and Pueraria mirifica at 40 mg./rat/day had significantly lower body weights, with significantly higher weights of the liver, uterus, adrenal glands and brain, than in the control group receiving Tripala only.($P<0.05$, $P<0.01$) (Table 9)

The blood analysis, table 10 shows that the hematocrit, hemoglobin, glucose, SGOT and SGPT of rats receiving only Tripala for 36 weeks were not significantly statistically different from the control group, receiving only distilled water (Table 10).

The groups receiving Tripala mixed with Pueraria mirifica at three different doses everyday for 36 weeks, showed that giving Tripala with Pueraria mirifica did not change the hematocrit, hemoglobin, glucose, SGOT and SGPT values significantly from the group receiving only Tripala (Table 11). However, rats receiving Tripala mixed with Pueraria mirifica at 4 and 40 mg./rat/day had significantly higher levels of SGPT than the group receiving only Tripala ($P<0.05$,$P<0.01$). Moreover, rats receiving Tripala with

Pueraria mirifica at 40 mg./rat/day had significantly lower [blood] levels of hematocrit, than the group receiving only Tripala ($P<0.05$).

Figure 1 shows that rats receiving Tripala weigh slightly more than the control group receiving only distilled water. It also showed that rats receiving Tripala with Pueraria mirifica at 0.4mg/rat/day weigh significantly more than rats receiving only Tripala. However, rats receiving Tripala with Pueraria mirifica at 4 and 40mg/rat/day weigh significantly less than rats receiving only Tripala especially rats receiving Tripala with Pueraria mirifica at 40mg/rat/day, which obviously have significantly lower weights than the group receiving Tripala.

Gross examination:

No significant changes between the control and the treated groups. Neither organ discoloration nor infiltrative lesions were observed.

Microscopic examination:

All the sampling tissue sections, both from the control and the treated groups, were within normal limits. Neither degenerative nor infiltrative lesions were observed. No significant changes referring to cell injury were noted and significantly identified among the animals.

Brain: A whole section of the brain, consisting of cerebrum, cerebellum, and medulla from both control and treated groups were submitted for microscopic review.

The cortical cellular arrangement is well preserved and the basal nuclei are unremarkable. Neither neuronal necrosis nor gliosis is observed. The ependyma is well aligned and the granulation tissue is intact. No significant alteration is discernable either in the control or in the treated animal.

Heart: A whole section of hearts containing both ventricles from all the animals were submitted for microscopic review

The endocardium was thin without mural thrombosis. The myocardium was well preserved without infarct or cellular infiltration. The epicardial fat is variable in amounts without fat necrosis and the epicardial surface is smooth. No pathologic condition was noted either in the control or the treated animals.

Liver: Unremarkable tissue appearances with intact hepatic architectures. The liver cells and the Kupffer cells were well preserved without necrosis, cholestasis or active inflammatory infiltration. There were minimal fatty changes, represented as minute fat droplets in some tissue sampling, both from the control and the treated groups, without significance.

Spleen: No Pathologic conditions: the splenic capsules were thin with uniform arrays of trabecular arteries and the white pulps. There were no definitive increased amounts or cellular constituents in the white pulp. Neither cellular destruction nor pigmentation was found in the red pulp. No fibrosis was seen.

Kidney: The whole cuts of both kidneys from all the animals were submitted for microscopic examination.

The renal cortices are rather congested but the renal capsules were intact. The glomeruli and the tubules are unremarkable. No definitive cellular injury such as cloudy swelling or necrosis was seen. There was no cellular infiltration in the tubuloiuterstitium. The renal medullar is composed of well-aligned tubules. No pathologic alterations were seen.

Adrenals: No pathologic conditions: the adrenal cortices and medullae were clearly discernable with unremarkable cellular appearance. The medullae cells were crowned with abundant cytoplasmic granules. The zona fasciculata of the adrenal cortices are well preserved. No cellular changes referring either hyperplasia or injury are included.

Pituitary glands: No pathologic conditions: both the anterior and posterior lobes are included together with prominent pars tubularis (intermedia). Most of the cells in the anterior lobes are chromophobes, which were rather large, containing granular cytoplasm. The acidophilic cells and the basophilic cells were present as small aggregates among the chromophobes without definitive cellular injury included.

Discussion

The toxicity study of Pueraria mirifica mixed with Tripala (the recommended formulation from the Ministry of Public Health) in female rats receiving 0.4 mg, 4.0mg and 40 mg was done using a single does administration of Pueraria mirifica to the rats. The rats were observed every 30 minutes for six hours and again at the 24th hour. As no abnormality was seen, the rats were continued to be treated with Pueraria mirifica for one month.

The data showed that Pueraria mirifica did not alter the body weight and the weight of the liver, kidney, heart, spleen, ovary, uterus, adrenal glands, brain, and pituitary gland significantly from the control group. This data supports the claim that Pueraria mirifica at three different doses does not have acute toxicity, and sub-acute toxicity or caused transformation of organs of the tested animals.

The result of the three months sub-chronic toxicity study of Tripala and Pueraria mirifica in female rats at three different doses showed that Pueraria mirifica at 40mg/rat/day made the weight of the liver, kidney, and heart significantly higher than in the control group. ($P<0.01$) This is probably a consequence of receiving Pueraria mirifica for certain periods of time; Pueraria mirifica increases the level of cholesterol (Anusorn 1989) and therefore, the liver might be responding to this change in order to control the excretory system, metabolism and vasoregulatory functions. This response could explain an increase in the liver weight. Secondly, this is probably due to receiving Pueraria mirifica for certain periods of time, steroids such as Beta-sitosterol in Pueraria mirifica might be affecting the absorption of cholesterol, causing the liver, which synthesizes cholesterol to work harder, to compensate for the lower cholesterol absorption. This leads to an increase in liver cells in order to support its extra work. This leads to a

significantly higher liver weight than the control group. This result corresponds with the report of Peter et al., (1997), stating that Phytosterols might inhibit cholesterol absorption and synthesizeation. Moreover, there are many kinds of flavonoids found in Pueraria mirifica. Herrera et al (1996), reported that flavonoids cause vasodilatation in the aorta rings of rats. In addition, Struckman, (1999) also found that flavonoids can be used for curing chronic venous insufficiency. From the actions of Phytosterols and flavonoids found in Pueraria mirifica, it can lower cholesterol, which is at the origin of problems related to blood vessels and it also could facilitate the function of blood vessels by allowing more blood flow into organs such as kidney and heart of the rats.

In addition, some chemicals in Pueraria mirifica might decrease cholesterol levels in blood vessels and at the same time expand it. This is probably the increased the blood flow to some organs such as the kidney and the heart in the rats. Since kidneys and the heart are sensitive to internal changes, it is possible that in three months, these organs were in a state of transformation to become significantly heavier than in the control group. Moreover, the study showed that weight of the brain of rats receiving Pueraria mirifica 40mg/rat/day is significantly greater than in the control group ($P<0.05$). This is probably because the blood circulation in the body was increased. Since the brain demands a lot of blood, an increase in blood circulation would probably increase the weight of the brain.

There was no difference in body weight and that of other organs between the control group and test group.

In pathological analysis of the liver, kidney, heart, brain, spleen, adrenal glands and pituitary gland, no abnormality in all organs was found. Pueraria mirifica mixed with Tripala given to rats at all doses should be safe over a three-month-period.

In the histopathological analysis, there was no sign of toxicity from Pueraria mirifica in the cells of internal organs of the rats. Therefore, Pueraria mirifica at these doses for three months is safe from any sub-acute toxicity.

The result of the 6 months sub-chronic toxicity study of Tripala and Pueraria mirifica in female rats at three different doses shows that only the weight of the liver in rats receiving 40mg/rat/day of Pueraria mirifica was significantly higher than in the control group ($P<0.05$). Stated earlier that in the three-month period, the liver of rats in this group worked more and weighted significantly more than the control group ($P<0.01$). However, after three months, the livers of the rats in this group began to recover, but weighed more than the control group. This made the weights of the livers at the end of the six-month-period ($P<0.05$) less significant than the treatment group in three-month-period ($P<0.01$). There was no significant difference in the body weight and the weight of other organs. In pathological analysis of the liver, kidney, heart, spleen, adrenal glands, brain, and pituitary gland, no abnormality in any organ was found. There was also no indication of chronic toxicity from Pueraria mirifica in all the groups. Therefore, Pueraria mirifica at these doses for six months is safe from any toxicity.

The results of the 9 months chronic toxicity study of Tripala and Pueraria mirifica in female rats at three different doses shows that the body weights and weights of organs of rats receiving Tripala and Pueraria mirifica at 0.4 and 4 mg/rat/day were not significantly different from the control group. Only rats receiving 40mg/rat/day

had significantly lower body weights and significantly higher liver, uterus, adrenal glands and brain weight greater than in the control group ($P<0.01$). The reason that the weight of these organs were significantly higher than in the control group was that the body weight of rats in this group at the end of the study, was significantly lower, than in the control group, making the percentage of the relative organ weights (organ weight/body weight) of many organs significantly higher than in the control group. However, pathological analysis of the liver, kidney, heart, spleen, adrenal glands, brain, and pituitary gland, showed that there was no abnormality in the cells of any organs indicating toxicity of Pueraria mirifica. Therefore, it may be said that Pueraria mirifica at 0.4 and 4 mg/rat/day is safe from chronic toxicity, while even though there were no abnormalities in the pathology of the groups that received 40mg/rat/day; the study of this group should be repeated to confirm the safety of long–term usage of Pueraria mirifica in high doses.

Conclusion

From this research, we can conclude that Pueraria mirifica at 0.4, 4.0 mixed with Tripala is safe from acute toxicity, sub-acute toxicity, chronic toxicity and sub-chronic toxicity in short term (1-3 months) and long term (6-9 months) use.

However, 40 mg/rat/day of Pueraria mirifica mixed with Tripala was safe in the short term (1-3 months) and in long-term usage (6-9 months) the study should be repeated to re-confirm the results and re-confirm the safety.

1 day (Acute toxicity)

This study shows that there was no acute toxicity found in rats receiving Pueraria mirifica mixed with Tripala at three different levels.

1 month (sub-acute toxicity)

The study shows that Pueraria mirifica at three doses does not affect body weight and organ weight of test rats and from the pathological analysis, there is no indication of toxicity in the organ cells.

3 months (sub-chronic toxicity)

The study shows that Pueraria mirifica at 0.4 and 4 mg/rat/day mixed with Tripala does not affect the body weight and the organ weight of the tested rats and from the pathological analysis, there was no indication of toxicity in the organ cells.

Pueraria mirifica at 40mg/rat/day increased the weight of the liver, kidney, heart, and brain. However, from the pathological analysis, there was no indication of toxicity in the organ cells.

6 month (sub-chronic toxicity)

The study shows that Pueraria mirifica at 0.4 and 4 mg/rat/day mixed with Tripala did not affect the body weight and the organ weight of the tested rats and from the pathological analysis, there was no indication of toxicity in the organ cells.

Pueraria mirifica at 40mg/rat/day increased the weights of the liver. However, from the pathological analysis, there was no indication of toxicity in the organ cells.

9 month (chronic toxicity)

The study shows that Pueraria mirifica at 0.4 and 4 mg/rat/day mixed with Tripala does not affect the body weight and the organ weight of the tested rats and from the pathological analysis, there was no indication of toxicity in the organ cells.

Pueraria mirifica at 40mg/rat/day decreased the body weight and increased the weight of the liver, uterus, adrenal glands, brain, and pituitary gland. However, from the pathological analysis, there was no indication of toxicity in the organ cells.

Figure 1 Changes of the body weight of rats receiving Pueraria mirifica at different doses for 6 months

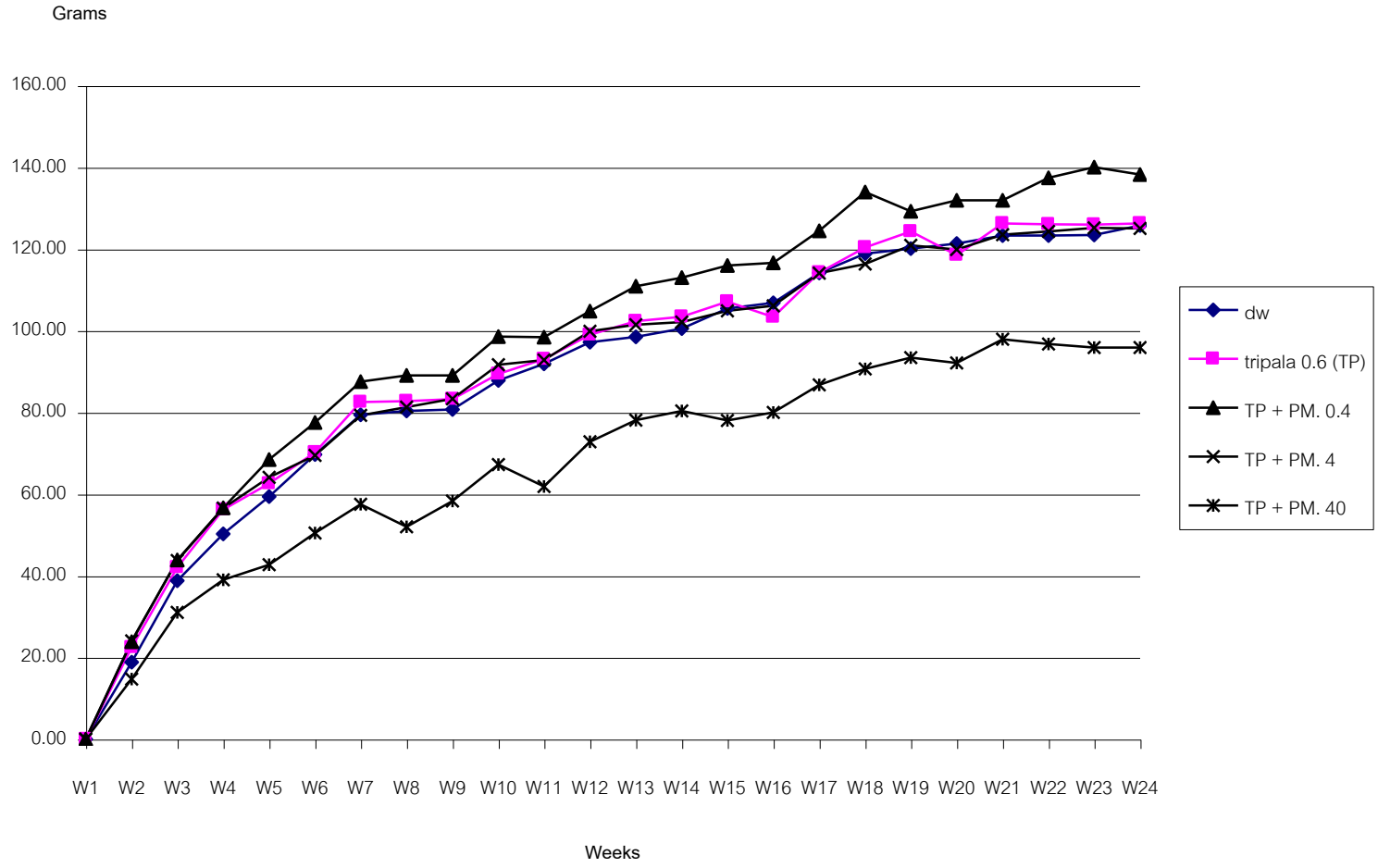

Table 1 Results of chronic toxicity in rats treated with Tripala (Emblic myrobalan, Terminalia chebula, and Belleric myrobalan) compared with group of rats treated with Pueraria mirifica mixed with Tripala (from Amphur Mae Loa) at different doses.

Group (1 ml./rat/once a day)	**Results of observation___minute after feed**								
	0	30	60	90	120	150	180	210	240
Distilled water	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Tripala (0.6 mg./rat/day)	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Tripala (0.6 mg./rat/day) mixed with Pueraria mirifica									
- 0.4 mg./rat/day	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
- 4 mg./rat/day	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
- 40 mg./rat/day	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Table 2 Result of sub-acute toxicity (4 weeks) in female rats treated with Tripala (Emblic myrobalan, Terminalia chebula and Beleric myrobalan) compared with the control group.

Subject of study	**roup**	
	Distilled water(n=6)	**Tripala (n=7)**
Body weight(g)	228.00 ±16.40	213.71 ±12.57
Organ weight (mg%)		
Liver	3751.57 ± 422.47	3962.19 ± 234.61
Kidney	665.53 ± 45.56	724.33 ± 68.01
Heart	349.95 ± 63.91	328.94 ± 36.25
Spleen	307.68 ± 47.81	319.05 ± 46.48
Ovary	41.97 ± 8.90	48.30 ±10.40
Uterus	169.35 ± 79.28	197.23 ± 97.44
Adrenal gland	35.33 ± 3.86	35.46 ± 3.31
Brain	811.52 ± 44.24	868.73 ± 60.52
Pituitary gland	3.87 ± 0.88	4.20 ± 0.29

Mean ± S.D.; Independent simple T-test

Table 3 Result of sub-acute toxicity (4 weeks) in female rats treated with Tripala compared with groups of female rats treated with Pueraria mirifica mixed with Tripala at different doses.

Subject of study	**Group** **ripala mixed with Pueraria mirifica _mg./rat/day**			
	Tripala (n=7)	**0.4(n=8)**	**4(n=8)**	**40(n=6)**
Body weight (g)	213.71 ±12.57	251.00 ±98.07	212.75 ±10.85	203.83 ±19.63
Organs weight (mg%)				
Liver	3962.19 ± 234.61	3660.70 ±879.77	4163.18 ±506.98	4793.96 ±789.73
Kidney	724.33 ± 68.01	622.46 ±144.66	752.36 ±81.17	786.61 ±50.45
Heart	328.94 ± 36.25	300.40 ± 77.63	334.95 ± 28.51	337.04 ± 25.17
Spleen	319.05 ± 46.48	266.12 ± 76.71	296.34 ± 47.73	319.91 ± 63.21
Ovary	48.30 ± 10.40	41.21 ± 12.05	41.99 ± 8.71	37.75 ± 2.69
Uterus	197.22 ± 97.44	225.14 ± 158.71	207.42 ±117.97	189.91± 28.86
Adrenal glands	35.46 ± 3.31	34.22 ± 9.08	37.94 ± 3.91	41.63 ± 10.42
Brain	868.73 ± 60.52	772.53 ± 172.02	862.21 ± 42.04	885.84 ± 103.59
Pituitary gland	4.20 ± 0.29	3.70 ± 0.90	4.41 ± 0.62	4.59 ± 0.44

Mean ± S.D. ; ANOVA ; LSD

Table 4 Results of sub-chronic toxicity (12 weeks) in female rats treated with Tripala compared with the control group

Subject of study	**roup**	
	Distilled water(n=8)	**Tripala (n=8)**
Body weight(g)	250.38 ± 17.10	±
Organ weight (mg%)		
Liver	2434.83 ± 266.40	2392.78 ± 197.39
Kidney	545.96 ± 66.06	516.02 ± 22.03
Heart	260.06 ± 19.89	272.20 ± 18.48
Spleen	243.39 ± 33.15	251.67 ± 31.53
Ovary	32.68 ± 10.56	33.20 ± 7.24
Uterus	184.94 ± 65.74	172.81 ± 77.37
Adrenal gland	28.66 ± 5.91	28.18 ± 3.68
Brain	753.87 ± 59.78	773.40 ± 39.01
Pituitary gland	4.64 ± 0.80	4.27 ± 0.52

Mean ± S.D.; Independent simple T-test

Table 5 Results of sub-chronic toxicity (12 weeks) in female rats treated with Tripala compared with groups of female rats treated with Tripala mixed with Pueraria mirifica at different doses.

Subject of study	**roup**			
	ripala mixed with Pueraria mirifica _mg./rat/day			
	Tripala (n=8)	**0.4(n=8)**	**4(n=8)**	**40(n=8)**
Body weight (g)	235.50 ± 12.22	246.13 ± 20.43	234.38 ± 14.22	228.13 ± 10.67
Organs weight (mg%)				
liver	2392.78 ±197.39	2470.97 ±217.91	2491.70 ±256.98	2908.22 ± 334.93**
Kidney	516.02 ± 22.03	561.02 ± 50.15*	552.63 ± 45.11	586.27 ± 42.30**
Heart	272.20 ± 18.48	277.21 ± 15.55	280.54 ± 20.13	299.80 ± 12.92**
Spleen	251.61± 31.53	255.61 ± 35.45	260.33 ± 27.70	279.61 ± 22.80
Ovary	33.20 ± 7.24	34.44 ± 3.72	35.86 ± 9.49	31.42 ± 3.80
Uterus	172.81 ± 77.37	218.42 ± 134.61	171.04 ± 54.41	212.31 ± 45.40
Adrenal glands	28.18 ± 3.68	27.25 ± 3.43	27.44 ± 4.26	30.94 ± 5.50
Brain	773.40 ± 39.01	749.48 ± 72.35	777.88 ± 37.54	825.53 ± 23.96**
Pituitary gland	4.27 ± 0.52	4.13 ± 0.58	3.94 ± 0.22	4.00 ± 0.98

Mean ± S.D.; ANOVA ; LSD *P<0.05, **P<0.01

Table 6 Results of sub-chronic toxicity (24 weeks) in female rats treated with Tripala compared with the control group.

Subject of study	**roup**	
	Distilled water(n=8)	**Tripala (n=8)**
Body weight(g)	265.50 ± 23.36	276.25 ± 19.45
Organ weight (mg%)		
Liver	3293.47 ± 335.92	2986.87 ± 323.28*
Kidney	574.83 ± 34.59	587.10 ± 55.30
Heart	269.94 ± 20.82	269.49 ± 14.82
Spleen	241.48 ± 33.44	233.93 ± 17.79
Ovary	31.38 ± 4.98	31.89 ± 6.96
Uterus	186.20 ± 45.70	162.29 ± 60.02
Adrenal glands	25.50 ± 3.44	22.87 ± 4.16
Brain	729.79 ± 87.31	716.94 ± 28.54
Pituitary gland	5.08 ± 0.72	4.64 ± 0.97

Mean ± S.D.; Independent simple T-test *P<0.05

Table 7 Results of chronic toxicity (24 weeks) in female rats treated with Tripala compared with groups of female rats treated with Pueraria mirifica mixed with Tripala at different doses.

Subject of study	**roup**			
	ueraria mirifica mixed with Tripala _mg./rat/day			
	Tripala (n=8)	**0.4(n=8)**	**4(n=8)**	**40(n=8)**
Body weight (g)	276.25 ± 19.45	286.50 ± 21.82	257.63 ± 23.58	256.38 ± 19.50
Organs weight (mg%)				
Liver	2986.87 ±323.28	2927.97 ±291.47	3347.94 ±452.04	3479.18 ± 426.43*
Kidney	587.10 ± 55.30	570.98 ± 63.26	607.75 ± 45.64	627.00 ± 54.19
Heart	269.49 ± 14.82	268.29 ± 13.29	286.26 ± 18.84	282.60 ± 21.89
Spleen	233.93 ± 17.79	226.81 ± 17.47	250.18 ± 37.91	231.45 ± 26.80
Ovary	31.89 ± 6.96	28.80 ± 3.14	28.97 ± 5.56	27.44 ± 4.59
Uterus	162.29 ± 60.02	145.22 ± 35.17	198.50 ± 64.35	222.83 ± 77.01
Adrenal glands	22.87 ± 4.16	21.64 ± 2.90	21.46 ± 3.79	23.83 ± 3.82
Brain	716.94 ± 28.54	667.33 ± 45.95	748.04 ± 72.46	751.70 ± 44.68
Pituitary gland	4.64 ± 0.97	4.15 ± 0.59	4.52 ± 0.63	4.20 ± 0.53

Mean ± S.D. ; ANOVA ; LSD ; *P<0.05

Table 8 Results of chronic toxicity (36 weeks) in female rats treated with Tripala compared with the control group

Subject of study	**roup**	
	distilled water (n=8)	**Tripala (n=8)**
Body weight(g)	284.22 ± 17.67	285.00 ± 29.91
Organ weight (mg%)		
Liver	2704.91 ± 338.56	2653.00 ± 430.93
Kidney	543.46 ± 37.48	535.05 ± 88.29
Heart	293.32 ± 22.47	287.28 ± 34.80
Spleen	244.29 ± 35.41	229.14 ± 29.08
Ovary	25.61 ± 9.55	30.18 ± 8.03
Uterus	196.18 ± 80.32	195.21 ± 66.77
Adrenal glands	23.86 ± 4.45	20.96 ± 4.18
Brain	703.43 ± 48.48	721.41 ± 74.67
Pituitary gland	4.53 ± 1.2	4.08 ± 1.10

Mean ± S.D.; Independent simple T-test

Table 9 Results of chronic toxicity (36 weeks) in female rats treated with Tripala compared with groups of female rats treated with Tripala mixed with Pueraria mirifica at different doses.

Subject of study	**roup** **ripala mixed with Pueraria mirifica _mg./rat/day**			
	Tripala (n=8)	**0.4(n=8)**	**4(n=8)**	**40(n=8)**
Body weight (g)	285.00 ± 29.91	306.13 ± 35.59	274.80 ± 20.61	255.57 ± 17.70*
Organs weight (mg%)				
Liver	2653.00 ±430.93	2542.34 ±349.45	2683.86 ±243.63	3504.65 ±281.10**
Kidney	535.05 ± 88.29	508.30 ± 55.34	552.16 ± 36.09	594.40 ± 47.36
Heart	287.28 ± 34.80	276.46 ± 31.44	287.77 ± 23.03	315.33 ± 26.48
Spleen	229.14 ± 29.08	207.44 ± 29.13	219.91 ± 25.13	287.29 ± 29.53
Ovary	30.18 ± 8.03	25.00 ± 7.04	29.45 ± 11.00	36.24 ± 11.43
Uterus	195.21 ± 66.77	139.10 ± 41.02	241.43 ± 88.67	261.50 ± 140.13**
Adrenal glands	20.96 ± 4.18	22.02 ± 5.70	20.39 ± 4.91	27.40 ± 2.91**
Brain	721.41 ± 74.67	559.40 ± 136.19	728.67 ± 67.45	861.05 ± 254.83**
Pituitary gland	4.08 ± 1.10	3.89 ± 0.63	4.18 ± 0.84	3.42 ± 1.05

Mean ± S.D. ; ANOVA ; LSD ; *P<0.05, **P<0.01

Table 10 Comparison of chemicals in serum of female rats treated with Tripala (Emblic myrobalan, Terminalia chebula, and Belleric myrobalan) and control group (36 weeks)

Subject of study	**Group**	
	Distilled water (n = 7)	**Tripala (n = 6)**
Haematocrit (%)	44.00 ± 1.63	45.17 ± 2.64
Haemoglobin (g/dl)	15.32 ± 1.51	15.23 ± 0.44
Glucose	136.86 ± 24.60	128.00 ± 11.97
SGOT	111.57 ± 19.51	109.73 ± 15.44
SGPT	35.26 ± 1.31	30.60 ± 4.24

Mean ± S.D. ; Independent simple T-test

Table 11 Comparison between haematocrit and some chemical constituents in blood in the serum of female rats treated with Pueraria mirifica at different doses and those of female rats treated with pure Tripala (36 weeks)

Subject of study	**roup** **ripala mixed with Pueraria mirifica _mg./rat/day**			
	Tripala (n=6)	**0.4(n=6)**	**4(n=5)**	**40(n=5)**
Hermatocrit (%)	45.17 ± 2.64	44.50 ± 2.88	45.20 ± 2.95	45.20 ± 2.95
Haemoglobin (g/dl)	16.23 ± 0.44	16.38 ± 0.58	15.20 ± 0.78	14.20 ± 2.42*
Glucose	128.00 ± 11.97	128.00 ± 15.17	138.20 ± 7.29	126.80 ± 32.31
SGOT	109.73 ± 15.44	115.92 ± 22.26	134.20 ± 42.94	119.58 ± 14.03
SGPT	30.60 ± 4.24	35.83 ± 6.65	39.20 ± 5.00*	44.62 ± 6.11**

ANOVA ; LSD

Mean ± S.D. ; *P<0.05, **P<0.01