

## REFERENCES

- Allred, C.D., Ju, Y.H., Allred, K.F., Chang, J. and Helferich, W.G. 2001. Dietary genistein stimulates growth of estrogen-dependent breast cancer tumors similar to that observed with genistein. *Carcinogenesis* 22: 1667-1673.
- Ashby, J., Odum, J., Paton, D., Lefevre, P.A., Beresford, N. and Sumpton, J.P. 2000. Re-evaluation of the first synthetic estrogen, 1-keto-1, 2, 3, 4-tetrahydrophenanthrene, and biphenol A using both the ovariectomized rat model used in 1933 and additional assays. *Toxicol Lett* 115: 231-238.
- Axelsson, M., Sijovall, J. and Gustafsson, B.E. 1984. Soya a dietary source of the nonsteroidal estrogen equol in man and animals. *J Endocrin* 102: 49-56.
- Balk, J.L., Whiteside, D.A., Naus, G., DeFerrari, E. and Roberts, J.M. 2002. A pilot study of the effects of phytoestrogen supplementation on postmenopausal endometrium. *J Soc Gynecol Investig* 9: 238-242.
- Benson, G.K., Cowie, A.T. and Hosking, Z.D. 1961. Mammogenic activity of miroestrol. *J Endocrin* 21: 401-409.
- Berkowitz, B.A. and Katzung, B.G. 2001. Basic and clinical evaluation of new drugs. In Katzung, B.G. (ed), *Basic Clin Pharmacol Toxicol*, McGraw-Hill New York: pp. 64-74.
- Boettiger, E.G. 1946. Changes in the glycogen and water contents of the rat uterus. *J Cell Physiol* 27: 9.
- Breithofer, A., Graumann, K., Scicchitano, M.S., Karatahnanis, S.K., Butt T.R. and Jungbauer, A. 1998. Regulation of human estrogen receptor by phytoestrogen in yeast and human cells. *J Steroid Biochem Mol Biol* 67: 421-429.
- Cain, J.C. 1960. Miroestrol: an estrogen from the plant *Pueraria mirifica*. *Nature* 158: 774-777.
- Carusi, D. 2000. Phytoestrogens as hormone replacement therapy: An evidence-based approach. *Clin Exp Obstet Gynecol* 7: 253-259.
- Chansakaow, S., Ishikawa, T. and Sekine, K. 2000<sup>a</sup>. Isoflavonoids from *Pueraria mirifica* and their estrogenic activity. *Planta Med* 66: 572-575.
- Chansakaow, S., Isikawa, T., Seki, H., Sekine (nee Yoshizawa), K., Okada, M. and Chaichantipyuth, C. 2000<sup>b</sup>. Identification of deoxymiroestrol as the actual

- rejuvenating principle of "Kwao Keur", *Pueraria mirifica*. The known miroestrol may be an artifact. *J Nat Prod* 63: 173-175.
- Chansri, K. 2002. *Using vaginal cytology to assess the estrogenic activity of phytoestrogen-rich herb*. Senior project. Chulalongkorn University.33p (in Thai).
- Chen, J., Xu, J. and Li, J. 1999. Effect of puerarin on fibrinolytic activity and lipid peroxide in patients with coronary heart disease. *Zhong Xi Yi Jie He Za Zhi* 19: 649-650.
- Cherdshewasart, W. 2003<sup>a</sup>. Biotechnology of Phytoestrogen-Rich; *P. mirifica*: III. Comparative proximate analysis. *The 29<sup>th</sup> Congress on Science and Technology of Thailand at Khonkaen University, 20-22 October 2003*. Thailand.
- Cherdshewasart, W. 2003<sup>b</sup>. Toxicity tests of a phytoestrogen-rich herb; *Pueraria mirifica*. *J Sci Res Chula* 28: 1-12.
- Cherdshewasart, W. Cheewasopit, W. and Picha, P. 2004<sup>a</sup>. The differatial anti-proliferation effect of white (*Pueraria mirifica*), red (*Butea superba*), and black (*Mucena colletti*) Kwao Krua plants on the growth of MCF-7 cells. *J Ethnopharm* 93: 255-260.
- Cherdshewasart, W. Cheewasopit, W. and Picha, P. 2004<sup>b</sup>. Anti-proliferation effects of the white(*Pueraria mirifica*), red (*Butea superba*) and Black (*Mucuna colletti*) Kwao Krua plant on the growth of HeLa cells. *J Sci Res Chula Univ* 29: 27-31
- Cherdshewasart, W., Malaivijitnond, S., Wattanasermkit, K., Panriansaen, R., Sittiwicheanwong, P., Temcharoen, P., Choung, S.Y. and Son, J.K. 2000. Toxicity tests of White Kwao Krua (*Pueraria mirifica*) cultivar Wichai-III in experimental animals. *The Fifth Joint Seminar Natural Medicines, NRCT-JSPS Core University system on Pharmaceutical Science*, Chulalongkorn University, Bangkok, Thailand.
- Cherdshewasart, W., Sompornpailin, K., and Recharoen, S. 1996. Tissue culture and field trial of *Pueraria mirifica* (Airy Shaw and Suvatabandhu). Principles regulating biosynthesis and storage of secondary products. *The Meeting of Phytochemical Society of Europe and the Martin-Luther-University*, Haale-Written. The Federal Republic of Germany.
- Chiechi, L.M., Putignano, G., Guerra, V., Schiavelli, M.P., Cisternino, A.M. and Carriero, C. 2003. The effect of soy rich diet on the vaginal epithelium in post menopause: a randomized double blind trial. *Maturitas* 45: 241-246.

- Chivapat, S., Chavalittumrong, P., Rattanajarasroj, S., Chuthaputti, A. and Panyamang, S. 2000. Toxicity study of *Pueraria mirifica* Airy Shaw et Sutavatabandhu. *Bull Med Sci* 42: 202-223 (in Thai).
- Connor, J.C., Cook, J.C., Craven, S.C., Van Pelt, C.S. and Obourn, J.D. 1996. An *in vivo* battery for identifying endocrine modulators that are estrogenic or dopamine receptors. *Fundam Appl Toxicol* 33: 182-195.
- Cook, J.W., Dodds, E.C. and Hewett, C.J. 1933. A synthetic oestrus-exciting compound. *Nature* 131: 56-57.
- Diel, P., Smolnikar, K., Schulz, T., Laudénbach-Leschowski, U., Michna, H. and Vollmer, G. 2001. Phytoestrogens and carcinogenesis-differential effects of genistein in experimental models of normal and malignant rat endometrium. *Hum Reprod* 16: 997-1006
- Dong, L.P. and Wang, T.Y. 1998. Effects of puerarin against glutamate excitotoxicity on cultured mouse cerebral cortical neurons. *Acta Pharmacol Sin* 19: 339-42
- Duan, H.J., Liu, S.X., Zhang, Y.J., Liu, Q.J., He, N. and Li, Y.M. 2004. Effects of puerarin on renal function, expressions of MMP-2 and TIMP-2 in diabetic rats. *Acta Pharmacol Sin* 39: 481-485.
- Duan, S., Li, Y.F. and Luo, X.L. 2000. Effect of puerarin on heart function and serum oxidized-LDL in the patients with chronic cardiac failure. *Hunan Yi Ke Da Xue Xue Bao* 25: 176-178.
- Eden, J.A. 2001. Managing the menopause: Phyto-oestrogens or hormone replacement therapy? *Ann Med* 31: 4-6.
- Enmark, E., Pelto-Huikko, M., Grandien, K., Lagercrantz, S., Lagercrantz, J., Fried, G., Nordenskjold, M. and Gustafsson, J.A. 1997. Human estrogen receptor  $\beta$ - gene structure, chromosomal localisation and expression pattern. *J Clin Endocrinol Metab* 82: 4258-4265.
- Franke, A.A., Custer, L.J., Cerna, C.M. and Narala, K.K. 1994. High-performance liquid chromatographic assay of isoflavonoids and coumestrol from human urine. *J Chromatogr B Biomed Appl* 662:47-60.
- Gercel-Taylor, C., Feitelson, A.K. and Taylor, D.D. 2004. Inhibitory effect of genistein and daidzein on ovarian cancer cell growth. *Anticancer Res* 24: 795-800.



- Gill, S., Sharpless, J.L., Rado, K. and Hall, J.E. 2002. Evidence that GnRH decreases with gonadal steroid feedback but increases with age in postmenopausal women. *J Clin Endocrinol Met* 87:2290-2296.
- Gustafsson, J.A. 1999. Estrogen receptor beta--a new dimension in estrogen mechanism of action. *J Endocrin* 163: 379-383.
- Hays, J., Ockene, J.K., Brunner, R.L., Kotchen, J.M., Manson, J.E., Patterson, R.E., Aragaki, A.K., Shumaker, S.A., Brzyski, R.G., LaCroix, A.Z. and Granek, I.A. Women's Health Initiative Investigators and Valanis, B.G. 2003. Effects of estrogen plus progestin on health-related quality of life. *N Engl J Med* 348, pp. 1835-1837.
- Hoyadom, M. 1971. Constituents of the tuberous roots of *Pueraria mirifica*. *Master's Thesis, Chulalongkorn University* 33 pp (in Thai).
- Humason, G.L. 1979. *Animal Tissue Techniques*. Sanfrancisco: Freeman.
- Ingham, J.L., Tahara, S., and Dziedzic, S.Z. 1986. A chemical investigation of *Pueraria mirifica* roots. *Z Naturforsch* 41c: 403-408.
- Ingham, J.L., Tahara, S., and Dziedzic, S.Z. 1988. Coumestan from the roots of *Pueraria mirifica* roots. *Z Naturforsch* 43c: 5-10.
- Ingham, J.L., Tahara, S., and Dziedzic, S.Z. 1989. Minor isoflavones from the roots of *Pueraria mirifica* roots. *Z Naturforsch* 44c: 724-726.
- Ishida, H., Uesugi, T., Hirai, K., Toda, T., Nukaya, H., Yokotsuka, K. and Tsuji, K. 1998. Prevention effects of plant isoflavones, daidzin and genistein on bone loss in ovariectomized rats fed with a calcium-deficient diet. *Biol Pharm Bull* 21: 62-66.
- Ji, E.S., Yin, J.X., Ma, H.J. and He, R.R. 2004. Effect of genistein on L-type calcium current in guinea pig ventricular myocytes. *Acta Pharmacol Sin* 56: 466-470.
- Johnson, M.H. and Everitt, B.J. 1995. *Essential Reproduction* 4<sup>th</sup> ed. London: *Blackwell science*. pp79-108.
- Jones, H.G.H. and Pope, G.S. 1960. A study of action of miroestrol and other oestrogen on the reproductive tract of the immature female mouse. *J Endocrin* 20: 229-235.
- Jones, H.E.H., Waynforth, H.B. and Pope, G.S. 1961. The effect of miroestrol on vaginal cornification, pituitary function and pregnancy in the rat. *J Endocrin* 22: 303-312.
- Joseph, A.H., Walter, R.F., Patricia, A.M. and Grace, A.W. 2000. Influence of Genotype and Environment on Isoflavone Contents of Soybean. *Crop Sci* 40: 48-51.



- Julsiri, M. and Cherdshewasart, W. 2003. Biotechnology of Phytoestrogen-Rich; *P. mirifica*: IV. Mutagenicity and antimutagenicity by Ames. *The 29<sup>th</sup> Congress on Science and Technology of Thailand at Khonkaen University*, 20-22 October. Thailand.
- Kashemsanta, M.C.L and Suvatabandhu, K. 1952. A new species of *Pueraria* (leguminosae) from Thailand, yielding and oestrogenic principle. *Kew Bull* 6: 263-266.
- Kashemsanta, M.C.L., Suvatabandhu, K., Popes, G. and Baetlett, S. 1957. Estrogen substance (miroestrol) from the tuberous root of *Pueraria mirifica*. *Proceeding 9<sup>th</sup> Pacific Science Congress Association*. 9:37(in Thai)
- Kerr, A. 1932. A reputed rejuvenator. *J Siam Soc* 8: 336-338.
- Khosla, S., Melton, L.J.<sup>3rd</sup>, Atkinson, E.J., O'Fallon, W.M., Klee, G.G. and Riggs, B.L.1998. Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. *J Clin Endocrinol Met* 83: 2266-2274.
- Kim, C.S., Lee, Y.S., Kim, J.S. and Hahn, Y.H. 2002. High performance liquid chromatographic analysis of isoflavones in soybean foods. *Korean Journal of Food Science Technology* 32: 25-30.
- Kim, H.Y., Hong, J.H., Kim, D.S., Kang, K.J., Han, S.B., Lee, E.J., Chung, H.W., Song, K.H., Sho, K.A., Kwack, S.J., Kim, S.S., Park, K.L., Kim, M.C., Kim, C.M. and Song, I.S. 2003. Isoflavone content and estrogenic activity in arrowroot *Puerariae Radix*. *Food Technol Biotechnol* 12: 29-35.
- Knight, D.C. and Eden, J. 1995. Phytoestrogen a short review. *Maturitas* 22: 167-175
- Kouki, T., Kishitake, M., Okamoto, M., Oosuka, I., Takebe, M. and Yamanouchi, K. 2003. Effect of neonatal treatment with phytoestrogens, genistein and daidzein, on sex difference in female rat brain function: estrous cycle and lordosis. *Horm Behav* 44: 140-145.
- Kuiper, G.J.M., Lemmen, L.G., Carlsson, B.O., Corton, J.C., Safe, S.H., Van Der Sang, P.T., Van Der Burg, B. and Gustafsson, J.A. 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor  $\beta$ . *Endocrinology* 139: 4252-4263.
- Kullavanijaya, P., Phaosavasdi, S., Taneepanichskul, S., Tannirandom, Y., Karnjanapitak, A and Pruksapong, C. 2003. The effects of the new Thai drug bill. *J Med Assoc Thai* 86:191-4.

- Kurzer, M.S. 2003. Phytoestrogen supplement use by women. *J Nutr* 133: 1983S- 1986S.
- Kushner, P.J., Hort, E., Shine, J., Baxter, J.D. and Greene, G.L. 1990. Construction of cell lines that express high levels of the human estrogen receptor and are killed by estrogens. *J Mol Endocrinol* 4: 1465-73
- Lamlertkitikul, S. and Chandeying, V. 2004. Efficacy and safety of *Pueraria mirifica* (Kwao Krua Khao) for the treatment of vasomotor symptoms in perimenopausal women: Phase II Study. *J Med Assoc Thai* 87:33-40.
- Lee, Y.S., Park, L.S., Cho, S.D., Son, J.K., Cherdshewasart, W. and Kang, K.S. 2002. Requirement of metabolic activation for estrogenic activity of *Pueraria mirifica*. *J Vet Sci* 3: 273-277.
- Li, S.M., Liu, B. and Chen, H.F. 1997. Effect of puerarin on plasma endothelin, renin activity and angiotensin II in patients with acute myocardial infraction. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 17: 339-341.
- Lin, R.C. and Li, T.K. 1998. Effects of isoflavones on alcohol pharmacokinetics and alcohol-drinking behavior in rats. *Am J Clin Nutr* 68: 1512S-1515S.
- Liu, J.M., Ma, L. and He, W.P. 2002. Therapeutic effect of puerarin therapy on sudden deafness. *Di Yi Jun Yi Da Xue Xue Bao* 22 : 1044-1053.
- Malaivijitnond, S., Jaroenporn, S., Wattanasermkit, K. and Cherdshewasart, W. 2003<sup>a</sup>. Biotechnology of phytoestrogen-rich; *Pueraria mirifica* : XII. Effects of *Pueraria mirifica* on fertility in mice. *The 29<sup>th</sup> Congress on Science and Technology of Thailand at Khonkaen University, 20-22 October 2003. Thailand.*
- Malaivijitnond, S., Keatthaipipat, P., Tansa, K., Jaroenporn, S., Ketsuwan, A., Watanabe, G., Taya, K. and Cherdshewasart, W. 2003<sup>b</sup>. Biotechnology of Phytoestrogen-rich; *Pueraria mirifica*: XIII. Sex differences in responses of anterior pituitary to *P. mirifica* phytoestrogens in rats. *The 29<sup>th</sup> Congress on Science and Technology of Thailand at Khonkaen University, 20-22 October 2003. Thailand.*
- Malaivijitnond, S., Kiatthaipipat, P., Cherdshewasart, W., Watanabe, G. and Taya, K. 2004. Different effect of *Pueraria mirifica*, and herb containing phytoestrogens, on LH and FSH secretion in gonadectomized female and male rats. *J Pharmacol Sci* 96: 428-435.

- Mandl, A.M. 1951. Cyclical changes in the vaginal smears of adult ovariectomized rats. *J Exp Biol* 28: 585-592.
- Markaverich, B.M., Webb B., Densmore C.L and Gregory, R.R. 1995. Effects of coumestrol on estrogen receptor function and uterine growth in ovariectomized rats. *Environ Health Perspect* 103: 574-589.
- McGarvey, C., Cates, P.S., Brooks, A.N., Swanson, I.A., Milligan, S.R., Coen, C.W. and O'Byrne, K.T. 2001. Phytoestrogens and gonadotropin-releasing hormone pulse generator activity and pituitary luteinizing hormone release in rat. *Endocrinology* 142: 1202-1208.
- Muangdet, N. and Anuntalabhojai, S. 1985. Effects of low dose of white Gwoon (*Pueraria mirifica* Shaw et Suvat.) on female Japanese quails. *J Sci Fac CMU* 13: 29-37 (in Thai).
- Muangman, V. and Cherdshewasart, W. 2001. Clinical trial of the phytoestrogen-rich herb, *Pueraria mirifica* as a crude drug in the treatment of symptoms in menopausal women. *Siriraj Hosp. Gazz.* 53: 300-309.
- Murkies, A.L., Wilcox, G. and Davis, S.R. 1998. Clinical review 92: Phytoestrogens. *J Clin Endocrinol Metab* 83: 297-303.
- Nikov, G.N., Hopkins, N.E., Boue, S. and Alworth, W.L. 2000. Interactions of dietary estrogens with human estrogen receptors and the effect on estrogen receptor-estrogen response element complex formation. *Environ Health Perspect* 108: 867-72.
- Nilanddhi, T., Kamthong, B., Isararena, K. and Shienghong, D. 1957. Constituents of the tuberous roots of *Pueraria mirifica*. *Z Naturforsch* 5c: 41.
- Nogowski, L. 1999. Effects of phytoestrogen-coumestrol on lipid and carbohydrate metabolism in young ovariectomized rats may be independent of its estrogenicity. *J Nutr* 10: 664-669.
- Norris, D.O. 1997. *Vertebrate Endocrinology*. 3<sup>rd</sup>. Academic press. San Diego. pp33-44.
- Okazaki, K., Okazaki, S., Nakamura, H., Kitamura, Y., Wakabayashi, S., Tsuda, T., Katsumata, T., Nishikawa, A. and Hirose, M. 2002. A repeated 28-day oral dose toxicity of genistein in rats, based on the 'Enhanced OECD test guideline 407' for screening endocrine-disrupting chemicals. *Arch Toxicol* 76: 553-559.



- Panriansaen, R. 2000. *Characterization of Pueraria mirifica populations from various parts of Thailand*. Master's Thesis, Chulalongkorn University. 116pp.
- Panriansaen, R and Cherdshewasart, W. 2003. Biotechnology of Phytoestrogen-Rich; *P. mirifica*: II. F1 analysis and the 2 clones of field grown plant. *The 29<sup>th</sup> Congress on Science and Technology of Thailand at Khonkaen University*, 20-22 October 2003 Thailand.
- Pisetpakasit, R.1976. *A Pharmaconostical study of Pueraria mirifica*. Master's thesis, Chulalongkorn University (in Thai).
- Price, K.R. and Fenwick, G.R. 1985. Naturally occurring oestrogens in foods- a review. *Food Addit Contam* 2: 73-106.
- Reel, J.R., Lamb, J.C., and Neal, B.H. 1996. Survey and assessment of mammalian estrogen biological assays for hazard characterization. *Fundam Appl Toxicol* 34: 288-305.
- Rhoades, R.A. and Pflanzer, R. 1996. *Human Physiology* Saunders College Publishing.
- Sang, H.F., Mei, Q.B., Xu, L.X., Wang, Q., Cheng, H. and Xiong, L.Z. 2004. Effect of puerarin on neural function and histopathological damages after transient spinal cord ischemia in rabbits. *Chin J Traumatol* 7:143-147.
- Santell, R.C., Chang, C.C., Nair M.G. and Helferich W.G. 1997. Dietary genistein exerts estrogenic effects upon the uterus, mammary gland and the hypothalamic/ pituitary axis in rats. *J Nutr* 127: 263-269.
- Schoeller, W., Dohn, M. and Hohweg, W. 1940. An estrogenic substance from the tubers of the Siamese vine, *Butea superba*. *Naturwissenschaften* 28: 252.
- Semler, D.E., In Shayne, C.G. and Chengelis, C.P.(eds). *Animal Models in Toxicology*. Academic Press. New York; pp 21-76.
- Shukla, S., Mathur, R and Prakash, A. 1987. Effect of butanolic extract of *Pueraria tuberosa* DC. on the oestrous cycles of adult rats. *Indian J Pharmac* 19: 49-53.
- Smitasiri, Y., Panjit, S., and Anuntalabhochai, S. 1989. Inhibition of lactation in lactating rats with *Pueraria mirifica* compared with estrogen. *J Sci Fac CMU* 16: 7-11.
- Soto, A.M., Sonnenschein, C., Chung, K.L., Fernandez, M.F., Olea, N. and Serrano, F.O. 1995. The E-Screen assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. *Environ Health Perspect* 103:113-122.

- Sriwatcharakul, S., Cherdshewasart, W. and Asawaprapa, P. 2003. Biotechnology of Phytoestrogen-Rich; *P. mirifica*: XVII. Field trial of *P. mirifica* for commercial demand. *The 29<sup>th</sup> Congress on Science and Technology of Thailand at Khonkaen University*, 20-22 October 2003. Thailand.
- Strauss, L., Makela, S., Joshi, S., Huhtaniemi, I. and Santi, R. 1998. Genistein exerts estrogenic-like effects in male mouse reproductive tract. *Mol Cell Endocrinol* 144: 83-93.
- Strobl, J.S. and Lippman, M.E. 1979. Prolonged retention of estradiol by human breast cancer cells in tissue culture. *Cancer Res* 39: 3319-3327.
- Stroheker, T., Chagnan, M-C., Pinner, M-F., Berges, R. and Canivenc-Lavier, M-C. 2003. Estrogenic effects of food wrap packaging xenoestrogens and flavonoids in female rats: a comparative study. *Reprod Toxicol* 17: 421-432.
- Subtang, S. 2002. *Comparative isoflavone HPLC fingerprints from The extracts of White Kwao Krua Pueraria mirifica in Thailand*. Master's Thesis, Chulalongkorn University.
- Subtang, S. and Cherdshewasart, W. 2003. Biotechnology of Phytoestrogen-Rich; *P. mirifica*: I. HPLC fingerprint and quantification of major isoflavonoids, *The 29<sup>th</sup> Congress on Science and Technology of Thailand at Khonkaen University*, 20-22 October 2003. Thailand.
- Sukhavachana, P. 1949. The comparison of the effects from *Pueraria mirifica* extract with oestrogenic Hormone. *J Med Assoc Thai* 3: 104-110 (in Thai).
- Suntara, L.A. 1931. *The Kwoa Krua Tuber Pamphlet*. Upatipong Printing. Chiangmai: 18pp (in Thai).
- Sutjit, W. and Cherdshewasart, W. 2003. Biotechnology of Phytoestrogen-Rich; *P. mirifica*: V. Antioxidant properties. *The 29<sup>th</sup> Congress on Science and Technology of Thailand at Khonkaen University*, 20-22 October 2003. Thailand.
- Terenius, L. 1971. The allen doisy test for estrogens reinvestigated. *Steroids* 17: 653-618
- Trisap, V., Cherdshewasart, W. and Picha, P. 2003. Biotechnology of Phytoestrogen-Rich; *P. mirifica*: VIII. Comparative proliferative and anti-proliferation effects on the growth of MCF-7 cells of *P. mirifica* from various sites. *The 29<sup>th</sup> Congress on Science and Technology of Thailand at Khonkaen University*, 20-22 October 2003. Thailand.

- Trisap, V., Cherdshewasart, W. and Picha, P. 2004. Comparative proliferative and antiproliferative effects on the growth of MCF-7 cells of *B. superba* collected from various sites. *ASIATOX III*, Bangkok, Thailand.
- Trisap, V. 2003. *Comparative proliferative and antiproliferative effects on the growth of MCF-7 cells of P. mirifica, B. superba and M. colletti collected from various sites*. Master's Thesis, Chulalongkorn University.
- Trisomboon, H., Malaivijitnond, S., Suzuki, J., Hamada, Y., Watanabe, G. and Taya, K. 2004<sup>a</sup>. Long-term treatment effects of *Pueraria mirifica* phytoestrogens on parathyroid hormone and calcium levels in aged menopausal cynomolgus monkeys. *J Reprod Dev* 50: 639-645.
- Trisomboon, H., Malaivijitnond, S., Watanabe, G. and Taya, K. 2004<sup>b</sup>. Estrogenic effect of *Pueraria mirifica* on the menstrual cycle and hormones related ovarian function in cyclic female cynomolgus monkeys. *J Pharmacol Sci* 94: 51-59.
- Tsuzumi, N. 1995. Effect of coumestrol on bone metabolism in organ culture. *Biol Pharm Bul* 18: 1012-1015.
- Turner. T. and Bagnara, S.1976. *Endocrinology USA*: WB. Saunders Company
- Vassilopoulou-Sellin, R. 2004. Hormone replacement therapy is not safe for breast cancer survivors. *Evid Based Med* 8: 224-226.
- Verma, S.P., Salomone, E. and Goldin, B. 1997. Curcumin and genistein plant natural products show synergistic inhibitory effects on the growth of human breast cancer MCF-7 cells induced by estrogenic pesticides. *Biochem Biophys Res Commun* 233: 692-696.
- Wang, C. and Kurzer, M.S. 1997. Phytoestrogen concentration determines effects on DNA synthesis in human breast cancer cells. *Nutr Cancer* 28: 236-247.
- Wilcox, G., Wahlqvist, M.L., Burger, H.G. and Medley, G. 1990. Oestrogenic effects of plants foods in postmenopausal women. *Br Med J* 301: 905-906.
- Wuttke, W., Jarry, H., Becker, T., Schultens, A., Christoffel, V., Gorkow, C. and Seidlova-Wuttke, D. 2003. Phytoestrogens: endocrine disrupter or replacement for hormone replacement therapy? *Maturitas* 44 (suppl.1): S9-S20.
- Xu, X., Hu, Y. and Ruan, Q. 2004. Effects of puerarin on learning-memory and amino acid transmitters of brain in ovariectomized mice. *Planta Med* 70: 627-631.



- Ye, H.Y., Qiu, F., Zeng, J., Yiao, X.S. and Lai, F. 2003. Effect of daidzein on antiarrhythmia. *Zhongguo Zhong Yao Za Zhi* 28: 853-856.
- Zava, D.T. and Duwe, G. 1997. Estrogenic and antiproliferative properties of genistein and other flavonoids in human breast cancer cells *in vitro*. *Nutr Cancer* 27: 31-40.
- Zhang, R., Li, Y. and Wang, W. 1988. Enhancement of immune function in mice fed high doses of soya daidzein. *Nutr Cancer* 29: 24-48.
- Zhu, J.H., Wang, X.X., Chen, J.Z., Shang, Y.P., Zhu, J.H., Guo, X.G. and Sun, J. 2004. Effects of puerarin on number and activity of endothelial progenitor cells from peripheral blood. *Acta Pharmacol Sin* 25: 1045-1051.
- Zhua, L., Jianga, Z.L., Krnjevi, B.K., Wanga, F.S. and Ye, J.H. 2003. Genistein directly blocks glycine receptors of rat neurons freshly isolated from the ventral tegmental area. *Neuropharmacology* 45: 270-280.



ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย

APPENDICES



ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย

## APPENDIX I

## HISTOLOGICAL STUDY

## 1. Chemicals

- Ethyl alcohol
- n-butyl alcohol
- Xylene
- Canada balsam
- Haematoxylin
- Eosin
- Paraffin
- Ammonia alum
- Glacial acetic acid
- 40% Formaldehyde
- Picric acid

## 2. Equipments

- Slide
- Cover glass
- Microtome
- Microtome blade
- Hot air oven
- Tissue floating bath
- Light microscope
- pH meter
- Hot plate

## 3. Procedures and methods

This study used the standard histological techniques (Humanson, 1979) using haematoxylin and eosin staining to study the structural features of the sections of the liver, ovary, uterus and mammary tumor. The processes were examined 5 steps as follows.



3.1 Fixation: All above tissues were fixed in 10% buffer formalin at least 24 hours after sacrifice. The tissues were transferred to newly 10% buffer formalin replacing the turbid one.

3.2 Dehydration: All tissues were cut into small pieces and transferred into ethyl alcohol as follows.

Step 1	90% ethanol	1 time	1 hour/time
Step 2	95% ethanol	2 time	6 hours/time
Step 3	N-butyl alcohol	1 time	1 hour/time
Step 4	Xylene	1 time	1 hour/time

3.3 Embedding: Then the tissue was impregnated in the hot air oven (58 °C). The tissue was placed in the embedding compound. Once the tissue had been infiltrated; it is placed into a mold and surrounded by wax and allowed to solidify into a block. The step wise were following.

Step 1	Xylene+molten wax (1:1)	1 time	½ hour/time
Step 2	Wax I	1 time	½ hour/time
Step 3	Wax II	1 time	1 hour/time
Step 4	Embedded and orientated in filtered wax.		

3.4 Sectioning: The blocks containing tissue were mounted onto a microtome. All tissue blocks were sectioned at 5 µm. Paraffin sections were mounted onto glass microscopic slides by egg albumin for further processing.

3.5 Hydration and staining: The glass microscopic slides containing paraffin sections were deparaffined and staining as follows.

1) 2 x 5 minutes	xylene
2) 1 x 3 minutes	n-butyl alcohol
3) 1 x 3 minutes	95% ethyl alcohol
4) 1 x 3 minutes	70% ethyl alcohol
5) 1 x 3 minutes	tap water
6) 10-12 minutes	Hematoxylin solution
7) 5-10 seconds	Acid alcohol
8) 10 minutes	running tap water
9) 1 x 3 minutes	70% ethyl alcohol

10) 1 x 3 minutes	90% ethyl alcohol
11) 3-5 minutes	Eosin staining solution
12) 15-30 seconds	95% ethyl alcohol
13) 1 x 5 minutes	n-butyl alcohol
14) 1 x 5 minutes	xylene

Then sides were mounted with cover slip by Canada balsam and laid slides flat while drying.

#### 4. Preparation of histological reagents

##### 1. 10% buffer formalin

- Formalin (40%)	100	ml
- Di-distilled water	900	ml
- Natrium dihydrogen phosphate-monohydrated ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ )	4	g
- Disodium hydrogen phosphate anhydrous ( $\text{Na}_2\text{HPO}_4$ )	6.5	g

These chemical substances were mixed together in the dark bottle, the solution was shaken until it was completely dissolved. This solution was stored at room temperature.

##### 2. Ehrlich's acid haematoxylin and eosin

- Haematoxylin	8	ml
- Absolute ethanol	400	ml
- Ammonium alum	8	g
- Di-distilled water	400	ml
- Glycerine	400	ml
- Glacial acetic acid	40	ml

Haematoxylin was dissolved in absolute ethanol in water bath at 40-50°C. When the solution was cool, it was filtered with filtered paper. Then ammonium alum was dissolved in warm di-distilled water. These two solution were mixed together, then glycerine and glacial acetic acid were added and stirred until these substances were completely dissolved. The solution need to expose to daylight to ripen for at least 6 weeks.

## 3. Eosin

- Eosin Y	0.5	g
- 95% Ethanol	100	ml

Eosin was dissolved in ethanol until the solution was completely dissolved and stored at room temperature.



ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย



## Appendix II

Table 5-1 The chemical isoflavone contents of *P. mirifica* tuber consist of puerarin, daidzin, genistein, daidzein and genistein analysis by HPLC (Subtang, 2002).

No.	Province	Puerarin (mean $\pm$ S.E.)	Daidzin (mean $\pm$ S.E.)	Genistin (mean $\pm$ S.E.)	Daidzein (mean $\pm$ S.E.)	Genistein (mean $\pm$ S.E.)	Total (mean $\pm$ S.E.)	Relative amount
1	Kanchanaburi	45.25 $\pm$ 1.11	50.24 $\pm$ 3.23	85.29 $\pm$ 1.23	13.92 $\pm$ 1.26	3.19 $\pm$ 0.29	198.29 $\pm$ 4.6	100
2	Lumphun	33.18 $\pm$ 0.92	28.35 $\pm$ 0.68	84.13 $\pm$ 0.54	8.59 $\pm$ 0.09	0.76 $\pm$ 0.36	155.00 $\pm$ 1.4	78.17
3	Sakon Nakhon	87.05 $\pm$ 0.79	11.48 $\pm$ 0.21	14.83 $\pm$ 0.22	4.78 $\pm$ 0.37	1.42 $\pm$ 0.14	119.57 $\pm$ 1.3	60.30
4	Mae Hong Son	36.99 $\pm$ 2.07	17.63 $\pm$ 1.74	55.44 $\pm$ 3.43	7.52 $\pm$ 1.27	1.54 $\pm$ 0.08	119.12 $\pm$ 6.5	60.07
5	Uthai thani	10.85 $\pm$ 1.01	21.70 $\pm$ 0.84	50.17 $\pm$ 3.57	16.48 $\pm$ 1.35	3.66 $\pm$ 0.16	102.86 $\pm$ 6.5	51.87
6	Sukhothai	14.12 $\pm$ 0.94	25.09 $\pm$ 1.50	51.43 $\pm$ 2.40	11.16 $\pm$ 0.85	0.73 $\pm$ 0.23	102.52 $\pm$ 5.3	51.70
7	Lumpang	34.65 $\pm$ 1.34	16.59 $\pm$ 0.08	33.30 $\pm$ 0.08	5.72 $\pm$ 0.09	1.54 $\pm$ 0.12	91.80 $\pm$ 1.72	46.29
8	Tak	29.06 $\pm$ 2.07	8.97 $\pm$ 0.99	43.86 $\pm$ 1.91	4.56 $\pm$ 0.32	1.15 $\pm$ 0.19	87.60 $\pm$ 4.87	44.18
9	Ratchaburi	8.85 $\pm$ 0.36	15.39 $\pm$ 0.79	51.15 $\pm$ 1.75	6.84 $\pm$ 0.53	2.54 $\pm$ 0.15	84.77 $\pm$ 2.67	42.37
10	Phitsanulok	35.24 $\pm$ 1.06	12.26 $\pm$ 0.13	26.53 $\pm$ 0.57	8.36 $\pm$ 0.23	1.63 $\pm$ 0.05	84.02 $\pm$ 1.91	42.75
11	Phetchaburi	13.19 $\pm$ 0.45	20.82 $\pm$ 1.78	37.56 $\pm$ 1.33	6.00 $\pm$ 0.24	1.13 $\pm$ 0.04	78.71 $\pm$ 3.15	39.69
12	Phrae	25.20 $\pm$ 1.54	10.55 $\pm$ 1.18	30.61 $\pm$ 0.81	5.45 $\pm$ 0.56	1.34 $\pm$ 0.14	73.16 $\pm$ 4.21	36.89
13	Lop Buri	19.50 $\pm$ 1.44	6.84 $\pm$ 0.09	39.47 $\pm$ 1.65	2.42 $\pm$ 0.79	0.98 $\pm$ 0.09	69.21 $\pm$ 3.77	34.90
14	Chaiyaphum	15.83 $\pm$ 2.43	12.91 $\pm$ 1.44	29.48 $\pm$ 2.33	7.02 $\pm$ 0.89	1.89 $\pm$ 0.42	67.13 $\pm$ 5.47	33.85
15	Uttharadith	30.25 $\pm$ 0.44	13.69 $\pm$ 0.21	10.27 $\pm$ 0.19	7.88 $\pm$ 0.18	0.87 $\pm$ 0.01	62.96 $\pm$ 1.03	31.75
16	Nakorn Sawan	13.34 $\pm$ 1.46	16.28 $\pm$ 1.64	27.71 $\pm$ 0.75	4.70 $\pm$ 0.37	0.72 $\pm$ 0.32	62.75 $\pm$ 3.24	31.64
17	Chiang Rai	20.02 $\pm$ 1.42	8.61 $\pm$ 1.12	29.58 $\pm$ 2.43	2.16 $\pm$ 0.08	0.50 $\pm$ 0.28	60.87 $\pm$ 3.30	30.70
18	Nong Bua Lam Phu	12.65 $\pm$ 2.42	11.91 $\pm$ 3.02	23.65 $\pm$ 2.14	7.46 $\pm$ 0.96	1.91 $\pm$ 0.25	57.58 $\pm$ 3.61	29.03
19	Phayoa	12.91 $\pm$ 0.99	8.46 $\pm$ 0.62	32.43 $\pm$ 1.35	3.03 $\pm$ 0.36	0.73 $\pm$ 0.30	57.56 $\pm$ 3.02	29.04
20	Chumphon	8.45 $\pm$ 0.22	7.38 $\pm$ 1.11	34.17 $\pm$ 4.81	2.64 $\pm$ 0.26	0.07 $\pm$ 0.06	52.70 $\pm$ 5.46	26.58
21	Phrachin Buri	12.42 $\pm$ 0.26	13.05 $\pm$ 0.65	16.69 $\pm$ 0.78	4.28 $\pm$ 0.56	0.51 $\pm$ 0.09	46.94 $\pm$ 1.12	23.67
22	Phetchabun	9.40 $\pm$ 0.46	10.48 $\pm$ 0.67	15.54 $\pm$ 1.61	8.11 $\pm$ 0.05	1.29 $\pm$ 0.02	44.83 $\pm$ 1.83	22.32
23	Nakorn Ratchasima	13.09 $\pm$ 0.77	5.61 $\pm$ 0.07	24.15 $\pm$ 1.42	1.20 $\pm$ 0.37	0.21 $\pm$ 0.19	44.27 $\pm$ 1.27	22.61
24	Kampaeng Phet	15.44 $\pm$ 1.14	7.01 $\pm$ 1.10	18.50 $\pm$ 4.45	2.31 $\pm$ 0.11	0.46 $\pm$ 0.08	43.71 $\pm$ 4.02	22.04
25	Nan	5.32 $\pm$ 0.44	2.36 $\pm$ 0.22	7.62 $\pm$ 1.36	3.31 $\pm$ 0.31	0.24 $\pm$ 0.04	18.85 $\pm$ 1.92	9.51
Mean		22.0 $\pm$ 11.77	14.96 $\pm$ 1.02	35.84 $\pm$ 2.04	6.13 $\pm$ 0.38	1.24 $\pm$ 0.09	80.79 $\pm$ 4.13	-

Table 5-2 *P. mirifica* collected from 24 provinces in Thailand showed variation of proliferative effect (1 ug/ml ) to MCF-7 cells (Trisap, 2003).

Provinces	1 ug/ml±SE	IC50
Phitsanulok	145.70±8.93	>1000 ug/ml
Nakhon Sawan	142.99±11.84	>1000 ug/ml
Phetchabun	135.67±2.11 <sup>b</sup>	>1000 ug/ml
Sukhothai	134.73±11.90	>1000 ug/ml
Nan	128.53±3.26	>1000 ug/ml
Chiang Rai	128.43±5.89	>1000 ug/ml
Ratchaburi	127.12±4.56	>1000 ug/ml
Mae Hong Son	127.04±8.81	>1000 ug/ml
Lamphun	125.83±3.26	>1000 ug/ml
Chumphon	123.69±7.05	>1000 ug/ml
Phrae	122.97±4.37	>1000 ug/ml
Chaiyaphum	120.62±2.74	>1000 ug/ml
Lop Buri	120.07±7.16	>1000 ug/ml
Phrachin Buri	119.69±10.27	>1000 ug/ml
Nong Bua Lam Phu	118.46±7.25	>1000 ug/ml
Phayoa	117.55±5.38	>1000 ug/ml
Tak	115.13±6.51	>1000 ug/ml
Sakon Nakhon	114.31±12.12	>1000 ug/ml
Uttharadith	112.92±3.77	>1000 ug/ml
Nakhon Ratchasima	112.68±4.87	1210.02ug/ml
kamphaeng phet	111.89±4.05	>1000 ug/ml
Lampang	111.61±5.45	>1000 ug/ml
Kanchanaburi	111.33±6.77	>1000 ug/ml
Phetchaburi	105.92±4.99	>1000 ug/ml
Means±SE	121.31±1.56	>1000 ug/ml

## APPENDIX III

## STATISTIC ANALYSIS

Table 5-3 The mean  $\pm$  SE day of appearance of cornified cells in rats after treated with *Pueraria mifca* collected from 3 regions during treatment period in dose 100 mg/kgBW/day.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean
					Lower Bound
North	15	9.9333	4.5272	1.1689	7.4263
NorthEast	4	10.2500	4.3493	2.1747	3.3292
Central	5	8.6000	4.9800	2.2271	2.4166
Total	24	9.7083	4.4280	.9039	7.8386

Table 5-4 Analysis of variance of Day of appearance cornified cell in 3 regions (Dose 100mg/kgBW/day)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.075	2	4.038	.191	.827
Within Groups	442.883	21	21.090		
Total	450.958	23			

Table 5-5 Multiple Comparisons; LSD -test of Day of appearance cornified cell in 3 regions (Dose 100mg/kgBW/day).

(I) code	(J) code	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
North	Northeast	-.32	2.584	.904	-5.69	5.06
	Central	1.33	2.371	.580	-3.60	6.27



Northeast	North	.32	2.584	.904	-5.06	5.69
	Central	1.65	3.081	.598	-4.76	8.06
Cen	North	-1.33	2.371	.580	-6.27	3.60
	Northeast	-1.65	3.081	.598	-8.06	4.76

Table 5-6 The mean  $\pm$  SE of day of appearance of cornified cells in rats after treated with *Pueraria mifica* collected from 3 regions during treatment period in dose 1000 mg/kgBW/day.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean
					Lower Bound
North	15	4.1333	1.3558	.3501	3.3825
NorthEast	4	4.7500	1.2583	.6292	2.7478
Central	5	4.0000	2.4495	1.0954	.9586
Total	24	4.2083	1.5598	.3184	3.5497

Table 5-7 Analysis of variance of Day of appearance cornified cell in 3 regions (Dose 1000 mg/kgBW/day)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.475	2	.737	.284	.755
Within Groups	54.483	21	2.594		
Total	55.958	23			

Table 5-8 Multiple Comparisons; LSD -test of Day of appearance cornified cell in 3 regions (Dose 1000 mg/kgBW/day).

(I) code	(J) code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
North	Northeast	-.62	.906	.504	-2.50	1.27
	Central	.13	.832	.874	-1.60	1.86
Northeast	North	.62	.906	.504	-1.27	2.50
	Central	.75	1.081	.495	-1.50	3.00
Central	North	-.13	.832	.874	-1.86	1.60
	Northeast	-.75	1.081	.495	-3.00	1.50

Table 5-9 The mean  $\pm$  SE of day of appearance of leucocyte cells in rats after treated with of *Pueraria mifca* collected from 3 regions, distilled water and estradiol valerate during posttreatment period (in dose 100mg/kgBW/day).

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean
					Lower Bound
North	15	2.1333	1.4573	.3763	1.3263
NorthEast	4	2.5000	2.3805	1.1902	-1.2879
Central	5	3.0000	2.3452	1.0488	8.804E-02
Total	24	2.3750	1.7647	.3602	1.6298

Table 5-10 Analysis of variance of Day of appearance leucocyte cell in 3 regions (Dose 100 mg/kgBW/day).

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.892	2	1.446	.442	.649
Within Groups	68.733	21	3.273		
Total	71.625	23			

Table 5- 11 LSD test of day of appearance leucocyte cells in 3 regions (dose 100 mg/kgBW/day).

(I) code	(J) code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
North	Northeast	-.37	1.018	.722	-2.48	1.75
	Central	-.87	.934	.364	-2.81	1.08
Northeast	North	.37	1.018	.722	-1.75	2.48
	Cen	-.50	1.214	.685	-3.02	2.02
Central	North	.87	.934	.364	-1.08	2.81
	Northeast	.50	1.214	.685	-2.02	3.02

Table 5-12 The mean  $\pm$  SE of day of appearance of leucocyte cells in rats after treated with of *Pueraria mifca* collected from 3 regions during posttreatment period (in dose 1000 mg/kgBW/day).

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean
					Lower Bound



North	15	5.2000	1.0142	.2619	4.6384
NorthEast	4	4.5000	1.2910	.6455	2.4457
Central	5	4.8000	1.3038	.5831	3.1811
Total	24	5.0000	1.1034	.2252	4.5341

Table 5-13 Analysis of variance of Day of appearance leucocyte cell in 3 regions (Dose 1000 mg/kgBW/day)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.800	2	.900	.721	.498
Within Groups	26.200	21	1.248		
Total	28.000	23			

Table 5-14 LSD test of day of appearance leucocyte cell in 3 regions (dose 1000 mg/kgBW/day).

(I) code	(J) code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
North	Northeast	.70	.629	.278	-.61	2.01
	Cen	.40	.577	.496	-.80	1.60
Northeast	North	-.70	.629	.278	-2.01	.61
	Cen	-.30	.749	.693	-1.86	1.26
Cen	North	-.40	.577	.496	-1.60	.80
	Northeast	.30	.749	.693	-1.26	1.86

Table 5-15 The mean  $\pm$  SE of the length of the appearance of cornified cells during the 14-day period of *P. mirifica* treatment and posttreatment also during the 14-day period of *P. mirifica* posttreatment( in the dose of 100 mg/kgBW /day).

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean
					Lower Bound
North	15	5.2667	5.9217	1.5290	1.9873
NorthEast	4	5.0000	6.0000	3.0000	-4.5473
Central	5	7.4000	7.0922	3.1718	-1.4062
Total	24	5.6667	5.9685	1.2183	3.1464

Table 5-16 Analysis of variance of the length of the appearance of cornified cells in the dose of 100 mg/kgBW /day.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19.200	2	9.600	.252	.780
Within Groups	800.133	21	38.102		
Total	819.333	23			

Table 5-17 LSD test of the length of the appearance of cornified cells in the dose of 100 mg/kgBW /day.

(I) code	(J) code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
North	Northeast	.27	3.474	.940	-6.96	7.49
	Central	-2.13	3.188	.511	-8.76	4.50

Northeast	North	-.27	3.474	.940	-7.49	6.96
	Central	-2.40	4.141	.568	-11.01	6.21
Central	North	2.13	3.188	.511	-4.50	8.76
	Northeast	2.40	4.141	.568	-6.21	11.01

Table 5-18 The mean  $\pm$  SE of the length of the appearance of cornified cells during the 14-day period of *P. mirifica* treatment and posttreatment also during the 14-day period of *P. mirifica* posttreatment( in the dose of 1000 mg/kgBW /day).

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean
					Lower Bound
North	15	14.3333	2.2254	.5746	13.1010
NorthEast	4	13.7500	2.5000	1.2500	9.7719
Central	5	14.6000	3.5071	1.5684	10.2453
Total	24	14.2917	2.4580	.5017	13.2538

Table 5-19 Analysis of variance of the length of the appearance of cornified cells in the dose of 1000 mg/kgBW /day.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.675	2	.837	.128	.880
Within Groups	137.283	21	6.537		
Total	138.958	23			

Table 5-20 LSD test of the length of the appearance of cornified cells in the dose of 1000 mg/kgBW /day.

(I) code	(J) code	Mean Difference	Std. Error	Sig.	95% Confidence Interval



		(I-J)				
					Lower Bound	Upper Bound
North	Northeast	.58	1.439	.689	-2.41	3.58
	Central	-.27	1.320	.842	-3.01	2.48
Northeast	North	-.58	1.439	.689	-3.58	2.41
	Central	-.85	1.715	.625	-4.42	2.72
Central	North	.27	1.320	.842	-2.48	3.01
	Northeast	.85	1.715	.625	-2.72	4.42

Table 5-21 Total day of appearance of cornified cells among three dose and the control group

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean
					Lower Bound
DW	5	.0000	.0000	.0000	.0000
D10	25	.0000	.0000	.0000	.0000
D100	25	5.9600	6.0241	1.2048	3.4734
D1000	25	14.3200	2.4104	.4821	13.3250
E2	5	19.2000	.4472	.2000	18.6447
Total	85	7.0941	7.3931	.8019	5.4995

Table 5-22 Analysis of variance of total day of appearance of cornified cells among three dose and the control group.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3580.047	4	895.012	70.808	.000
Within Groups	1011.200	80	12.640		
Total	4591.247	84			

Table 5-23 LSD-test of total day of appearance of cornified cells among three dose and the control group.

(I) code	(J) code	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Dw	D10	.0000	1.7417	1.000	-3.4661	3.4661
	D100	-5.9600*	1.7417	.001	-9.4261	-2.4939
	D1000	-14.3200*	1.7417	.000	-17.7861	-10.8539
	E2	-19.2000*	2.2486	.000	-23.6748	-14.7252
D10	DW	.0000	1.7417	1.000	-3.4661	3.4661
	D100	-5.9600*	1.0056	.000	-7.9612	-3.9588
	D1000	-14.3200*	1.0056	.000	-16.3212	-12.3188
	E2	-19.2000*	1.7417	.000	-22.6661	-15.7339
D100	DW	5.9600*	1.7417	.001	2.4939	9.4261
	D10	5.9600*	1.0056	.000	3.9588	7.9612
	D1000	-8.3600*	1.0056	.000	-10.3612	-6.3588
	E2	-13.2400*	1.7417	.000	-16.7061	-9.7739
D1000	DW	14.3200*	1.7417	.000	10.8539	17.7861
	D10	14.3200*	1.0056	.000	12.3188	16.3212
	D100	8.3600*	1.0056	.000	6.3588	10.3612
	E2	-4.8800*	1.7417	.006	-8.3461	-1.4139
E2	DW	19.2000*	2.2486	.000	14.7252	23.6748
	D10	19.2000*	1.7417	.000	15.7339	22.6661
	D100	13.2400*	1.7417	.000	9.7739	16.7061
	D1000	4.8800*	1.7417	.006	1.4139	8.3461

\* The mean difference is significant at the .05 level.

Table 5-24 Total Cornified day of rats were treated with *P. mirifica* 25 provinces dosage 1,000 mg/kgBW/day.

Provinces	Total cornified day $\pm$ SE
Chaing Rai	17 $\pm$ 0.63
Mae Hong Son	15.2 $\pm$ 0.74
Phayoa	11.8 $\pm$ 0.75
Nan	14 $\pm$ 0.63
Lumpang	14 $\pm$ 1.85
Phrae	14 $\pm$ 0
Lumphun	13 $\pm$ 0.4
Uttharadith	8.6 $\pm$ 0.89
Sukhothai	15 $\pm$ 0.4
Tak	15.6 $\pm$ 0.8
Phitsanulok	16.8 $\pm$ 0.4
Phetchabun	13.8 $\pm$ 0.4
Kampaeng Phet	15.8 $\pm$ 0.4
Nakorn Sawan	14.8 $\pm$ 0.4
Uthai thani	14.6 $\pm$ 0.8
Sakon Nakhon	11 $\pm$ 0.63
Nong Bua Lam Phu	13 + 0
Chaiyaphum	14.2 $\pm$ 0.4
Nakorn Ratchasima	16.8 $\pm$ 0.4
Lop Buri	14.8 $\pm$ 0.4
Kanchanaburi	18 $\pm$ 0
Phrachin Buri	14 $\pm$ 0.63
Ratchaburi	16.4 $\pm$ 0.8
Phetchaburi	9.6 $\pm$ 0.8
Chumphon	15.2 $\pm$ 0.4



Table 5-25 The body weight of rats in the control group on day 1 to day 42.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Day 1	5	239.2000	6.0581	2.7092	231.6779	246.7221
Day 7	5	245.8000	3.7683	1.6852	241.1211	250.4789
Day14	5	249.0000	5.5678	2.4900	242.0867	255.9133
Day 21	5	255.4000	6.2690	2.8036	247.6160	263.1840
Day 28	5	263.4000	9.7365	4.3543	251.3105	275.4895
Day 35	5	271.8000	10.8490	4.8518	258.3292	285.2708
Day 42	5	281.0000	7.9687	3.5637	271.1056	290.8944
Total	35	257.9429	15.6072	2.6381	252.5816	263.3041

Table 5-26 Analysis of variance of body weight of rats in negative control group.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6693.086	6	1115.514	19.659	.000
Within Groups	1588.800	28	56.743		
Total	8281.886	34			

Table 5-27 LSD test of body weight of rats in negative control group.

(I) code	(J) code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Day1	Day7	-6.6000	4.7642	.177	-16.3589	3.1589
	Day14	-9.8000*	4.7642	.049	-19.5589	-4.1077E-02

	Day21	-16.2000*	4.7642	.002	-25.9589	-6.4411
	Day28	-24.2000*	4.7642	.000	-33.9589	-14.4411
	Day35	-32.6000*	4.7642	.000	-42.3589	-22.8411
	Day42	-41.8000*	4.7642	.000	-51.5589	-32.0411
Day7	Day1	6.6000	4.7642	.177	-3.1589	16.3589
	Day14	-3.2000	4.7642	.507	-12.9589	6.5589
	Day21	-9.6000	4.7642	.054	-19.3589	.1589
	Day28	-17.6000*	4.7642	.001	-27.3589	-7.8411
	Day35	-26.0000*	4.7642	.000	-35.7589	-16.2411
	Day42	-35.2000*	4.7642	.000	-44.9589	-25.4411
Day14	Day1	9.8000*	4.7642	.049	4.108E-02	19.5589
	Day7	3.2000	4.7642	.507	-6.5589	12.9589
	Day21	-6.4000	4.7642	.190	-16.1589	3.3589
	Day28	-14.4000*	4.7642	.005	-24.1589	-4.6411
	Day35	-22.8000*	4.7642	.000	-32.5589	-13.0411
	Day42	-32.0000*	4.7642	.000	-41.7589	-22.2411
Day21	Day1	16.2000*	4.7642	.002	6.4411	25.9589
	Day7	9.6000	4.7642	.054	-.1589	19.3589
	Day14	6.4000	4.7642	.190	-3.3589	16.1589
	Day28	-8.0000	4.7642	.104	-17.7589	1.7589
	Day35	-16.4000*	4.7642	.002	-26.1589	-6.6411
	Day42	-25.6000*	4.7642	.000	-35.3589	-15.8411
Day28	Day1	24.2000*	4.7642	.000	14.4411	33.9589
	Day7	17.6000*	4.7642	.001	7.8411	27.3589
	Day14	14.4000*	4.7642	.005	4.6411	24.1589
	Day21	8.0000	4.7642	.104	-1.7589	17.7589
	Day35	-8.4000	4.7642	.089	-18.1589	1.3589
	Day42	-17.6000*	4.7642	.001	-27.3589	-7.8411
Day35	Day1	32.6000*	4.7642	.000	22.8411	42.3589
	Day7	26.0000*	4.7642	.000	16.2411	35.7589

	Day14	22.8000*	4.7642	.000	13.0411	32.5589
	Day21	16.4000*	4.7642	.002	6.6411	26.1589
	Day28	8.4000	4.7642	.089	-1.3589	18.1589
	Day42	-9.2000	4.7642	.064	-18.9589	.5589
Day42	Day1	41.8000*	4.7642	.000	32.0411	51.5589
	Day7	35.2000*	4.7642	.000	25.4411	44.9589
	Day14	32.0000*	4.7642	.000	22.2411	41.7589
	Day21	25.6000*	4.7642	.000	15.8411	35.3589
	Day28	17.6000*	4.7642	.001	7.8411	27.3589
	Day35	9.2000	4.7642	.064	-.5589	18.9589

\*The mean difference is significant at the .05 level.

Table 5-28 The body weight of rats in the dose of 10 mg/kgBW/day on day 1 to day 42.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Day 1	25	239.2080	3.3199	.6640	237.8376	240.5784
Day 7	25	244.1680	3.4257	.6851	242.7539	245.5821
Day14	25	250.1360	3.4505	.6901	248.7117	251.5603
Day 21	25	256.2640	2.8901	.5780	255.0710	257.4570
Day 28	25	260.9840	3.0287	.6057	259.7338	262.2342
Day 35	25	266.2400	3.6597	.7319	264.7294	267.7506
Day 42	25	273.2560	3.6844	.7369	271.7351	274.7769
Total	175	255.7509	11.7410	.8875	253.9991	257.5026



Table 5-29 Analysis of variance of body weight of rats in the dose of 10 mg/kgBW/day.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	22086.41 4	6	3681.069	325.525	.000
Within Groups	1899.763	168	11.308		
Total	23986.17 7	174			

Table 5-30 LSD test of body weight of rats in the dose of 10 mg/kgBW/day.

(I) CODE	(J) CODE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Day1	Day7	-4.9600*	.9511	.000	-6.8377	-3.0823
	Day14	-10.9280*	.9511	.000	-12.8057	-9.0503
	Day21	-17.0560*	.9511	.000	-18.9337	-15.1783
	Day28	-21.7760*	.9511	.000	-23.6537	-19.8983
	Day35	-27.0320*	.9511	.000	-28.9097	-25.1543
	Day42	-34.0480*	.9511	.000	-35.9257	-32.1703
Day7	Day1	4.9600*	.9511	.000	3.0823	6.8377
	Day14	-5.9680*	.9511	.000	-7.8457	-4.0903
	Day21	-12.0960*	.9511	.000	-13.9737	-10.2183
	Day28	-16.8160*	.9511	.000	-18.6937	-14.9383
	Day35	-22.0720*	.9511	.000	-23.9497	-20.1943
	Day42	-29.0880*	.9511	.000	-30.9657	-27.2103
Day14	Day1	10.9280*	.9511	.000	9.0503	12.8057
	Day7	5.9680*	.9511	.000	4.0903	7.8457
	Day21	-6.1280*	.9511	.000	-8.0057	-4.2503

	Day28	-10.8480*	.9511	.000	-12.7257	-8.9703
	Day35	-16.1040*	.9511	.000	-17.9817	-14.2263
	Day42	-23.1200*	.9511	.000	-24.9977	-21.2423
Day21	Day1	17.0560*	.9511	.000	15.1783	18.9337
	Day7	12.0960*	.9511	.000	10.2183	13.9737
	Day14	6.1280*	.9511	.000	4.2503	8.0057
	Day28	-4.7200*	.9511	.000	-6.5977	-2.8423
	Day35	-9.9760*	.9511	.000	-11.8537	-8.0983
	Day42	-16.9920*	.9511	.000	-18.8697	-15.1143
Day28	Day1	21.7760*	.9511	.000	19.8983	23.6537
	Day7	16.8160*	.9511	.000	14.9383	18.6937
	Day14	10.8480*	.9511	.000	8.9703	12.7257
	Day21	4.7200*	.9511	.000	2.8423	6.5977
	Day35	-5.2560*	.9511	.000	-7.1337	-3.3783
	Day42	-12.2720*	.9511	.000	-14.1497	-10.3943
Day35	Day1	27.0320*	.9511	.000	25.1543	28.9097
	Day7	22.0720*	.9511	.000	20.1943	23.9497
	Day14	16.1040*	.9511	.000	14.2263	17.9817
	Day21	9.9760*	.9511	.000	8.0983	11.8537
	Day28	5.2560*	.9511	.000	3.3783	7.1337
	Day42	-7.0160*	.9511	.000	-8.8937	-5.1383
Day42	Day1	34.0480*	.9511	.000	32.1703	35.9257
	Day7	29.0880*	.9511	.000	27.2103	30.9657
	Day14	23.1200*	.9511	.000	21.2423	24.9977
	Day21	16.9920*	.9511	.000	15.1143	18.8697
	Day28	12.2720*	.9511	.000	10.3943	14.1497
	Day35	7.0160*	.9511	.000	5.1383	8.8937

\* The mean difference is significant at the .05 level.

Table 5-31 The body weight of rats in the dose of 100 mg/kgBW/day on day 1 to day 42.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Day 1	25	239.9520	4.2183	.8437	238.2108	241.6932
Day 7	25	244.4960	4.3875	.8775	242.6849	246.3071
Day14	25	248.2000	4.6929	.9386	246.2629	250.1371
Day 21	25	246.0320	4.8366	.9673	244.0356	248.0284
Day 28	25	247.8320	4.5701	.9140	245.9456	249.7184
Day 35	25	250.8880	5.0313	1.0063	248.8112	252.9648
Day 42	25	253.6720	5.4918	1.0984	251.4051	255.9389
Total	175	247.2960	6.2342	.4713	246.3659	248.2261

Table 5-32 Analysis of variance of body weight of rats in the dose of 100 mg/kgBW/day.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2950.810	6	491.802	21.676	.000
Within Groups	3811.658	168	22.688		
Total	6762.467	174			

Table 5-33 LSD test of body weight of rats in the dose of 100 mg/kgBW/day.

(I) CODE	(J) CODE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Day1	Day7	-4.5440*	1.3472	.001	-7.2037	-1.8843



	Day14	-8.2480*	1.3472	.000	-10.9077	-5.5883
	Day21	-6.0800*	1.3472	.000	-8.7397	-3.4203
	Day28	-7.8800*	1.3472	.000	-10.5397	-5.2203
	Day35	-10.9360*	1.3472	.000	-13.5957	-8.2763
	Day42	-13.7200*	1.3472	.000	-16.3797	-11.0603
Day7	Day1	4.5440*	1.3472	.001	1.8843	7.2037
	Day14	-3.7040*	1.3472	.007	-6.3637	-1.0443
	Day21	-1.5360	1.3472	.256	-4.1957	1.1237
	Day28	-3.3360*	1.3472	.014	-5.9957	-.6763
	Day35	-6.3920*	1.3472	.000	-9.0517	-3.7323
	Day42	-9.1760*	1.3472	.000	-11.8357	-6.5163
Day14	Day1	8.2480*	1.3472	.000	5.5883	10.9077
	Day7	3.7040*	1.3472	.007	1.0443	6.3637
	Day21	2.1680	1.3472	.109	-.4917	4.8277
	Day28	.3680	1.3472	.785	-2.2917	3.0277
	Day35	-2.6880*	1.3472	.048	-5.3477	-2.8285E-02
	Day42	-5.4720*	1.3472	.000	-8.1317	-2.8123
Day21	Day1	6.0800*	1.3472	.000	3.4203	8.7397
	Day7	1.5360	1.3472	.256	-1.1237	4.1957
	Day14	-2.1680	1.3472	.109	-4.8277	.4917
	Day28	-1.8000	1.3472	.183	-4.4597	.8597
	Day35	-4.8560*	1.3472	.000	-7.5157	-2.1963
	Day42	-7.6400*	1.3472	.000	-10.2997	-4.9803
Day28	Day1	7.8800*	1.3472	.000	5.2203	10.5397
	Day7	3.3360*	1.3472	.014	.6763	5.9957
	Day14	-.3680	1.3472	.785	-3.0277	2.2917
	Day21	1.8000	1.3472	.183	-.8597	4.4597
	Day35	-3.0560*	1.3472	.025	-5.7157	-.3963
	Day42	-5.8400*	1.3472	.000	-8.4997	-3.1803

Day35	Day1	10.9360*	1.3472	.000	8.2763	13.5957
	Day7	6.3920*	1.3472	.000	3.7323	9.0517
	Day14	2.6880*	1.3472	.048	2.828E-02	5.3477
	Day21	4.8560*	1.3472	.000	2.1963	7.5157
	Day28	3.0560*	1.3472	.025	.3963	5.7157
	Day42	-2.7840*	1.3472	.040	-5.4437	-.1243
Day42	Day1	13.7200*	1.3472	.000	11.0603	16.3797
	Day7	9.1760*	1.3472	.000	6.5163	11.8357
	Day14	5.4720*	1.3472	.000	2.8123	8.1317
	Day21	7.6400*	1.3472	.000	4.9803	10.2997
	Day28	5.8400*	1.3472	.000	3.1803	8.4997
	Day35	2.7840*	1.3472	.040	.1243	5.4437

\* The mean difference is sig\*nificant at the .05 level.

Table 5-34 The body weight of rats in the dose of 1000 mg/kgBW/day on day 1 to day 42.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Day 1	25	241.5600	4.1697	.8339	239.8388	243.2812
Day 7	25	245.8560	3.7579	.7516	244.3048	247.4072
Day14	25	243.2320	3.0799	.6160	241.9607	244.5033
Day 21	25	241.4800	3.4088	.6818	240.0729	242.8871
Day 28	25	244.1600	3.6815	.7363	242.6404	245.6796
Day 35	25	246.3840	3.2741	.6548	245.0325	247.7355
Day 42	25	248.2000	3.1969	.6394	246.8804	249.5196
Total	175	244.4103	4.1890	.3167	243.7853	245.0353

Table 5-35 Analysis of variance of body weight of rats in the dose of 1000 mg/kgBW/day.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	962.732	6	160.455	12.894	.000
Within Groups	2090.570	168	12.444		
Total	3053.301	174			

Table 5-36 LSD test of body weight of rats in the dose of 1000 mg/kgBW/day.

(I) code	(J) code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Day1	Day7	-4.2960*	.9978	.000	-6.2657	-2.3263
	Day14	-1.6720	.9978	.096	-3.6417	.2977
	Day21	8.000E-02	.9978	.936	-1.8897	2.0497
	Day28	-2.6000*	.9978	.010	-4.5697	-.6303
	Day35	-4.8240*	.9978	.000	-6.7937	-2.8543
Day7	Day1	4.2960*	.9978	.000	2.3263	6.2657
	Day14	2.6240*	.9978	.009	.6543	4.5937
	Day21	4.3760*	.9978	.000	2.4063	6.3457
	Day28	1.6960	.9978	.091	-.2737	3.6657
	Day35	-.5280	.9978	.597	-2.4977	1.4417
Day14	Day1	1.6720	.9978	.096	-.2977	3.6417
	Day7	-2.6240*	.9978	.009	-4.5937	-.6543
	Day21	1.7520	.9978	.081	-.2177	3.7217
	Day28	-.9280	.9978	.354	-2.8977	1.0417



	Day35	-3.1520*	.9978	.002	-5.1217	-1.1823
	Day42	-4.9680*	.9978	.000	-6.9377	-2.9983
Day21	Day1	-8.0000E-02	.9978	.936	-2.0497	1.8897
	Day7	-4.3760*	.9978	.000	-6.3457	-2.4063
	Day14	-1.7520	.9978	.081	-3.7217	.2177
	Day28	-2.6800*	.9978	.008	-4.6497	-.7103
	Day35	-4.9040*	.9978	.000	-6.8737	-2.9343
	Day42	-6.7200*	.9978	.000	-8.6897	-4.7503
Day28	Day1	2.6000*	.9978	.010	.6303	4.5697
	Day7	-1.6960	.9978	.091	-3.6657	.2737
	Day14	.9280	.9978	.354	-1.0417	2.8977
	Day21	2.6800*	.9978	.008	.7103	4.6497
	Day35	-2.2240*	.9978	.027	-4.1937	-.2543
	Day42	-4.0400*	.9978	.000	-6.0097	-2.0703
Day35	Day1	4.8240*	.9978	.000	2.8543	6.7937
	Day7	.5280	.9978	.597	-1.4417	2.4977
	Day14	3.1520*	.9978	.002	1.1823	5.1217
	Day21	4.9040*	.9978	.000	2.9343	6.8737
	Day28	2.2240	.9978	.027	.2543	4.1937
	Day42	-1.8160	.9978	.071	-3.7857	.1537
Day42	Day1	6.6400*	.9978	.000	4.6703	8.6097
	Day7	2.3440*	.9978	.020	.3743	4.3137
	Day14	4.9680*	.9978	.000	2.9983	6.9377
	Day21	6.7200*	.9978	.000	4.7503	8.6897
	Day28	4.0400*	.9978	.000	2.0703	6.0097
	Day35	1.8160*	.9978	.071	-.1537	3.7857

\* The mean difference is significant at the .05 level.

Table 5- 37 The uterus weight compare among three dose and control group.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean
					Lower Bound
D10	125	.1278	3.192E-02	2.855E-03	.1222
D100	125	.1542	3.441E-02	3.078E-03	.1481
D1000	125	.1700	4.182E-02	3.741E-03	.1626
DW	5	.1334	1.180E-02	5.278E-03	.1187
E2	5	.2694	6.735E-02	3.012E-02	.1858
Total	385	.1520	4.250E-02	2.166E-03	.1477

Table 5-38 Analysis of variance of uterus weight among three dose and control group

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.185	4	4.618E-02	34.493	.000
Within Groups	.509	380	1.339E-03		
Total	.693	384			

Table 5-39 LSD test of uterus weight among three dose and control group.

(I) code	(J) code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
D10	D100	-2.6374E-02*	4.628E-03	.000	-3.5474E-02	-1.7274E-02
	D1000	-4.2154E-02*	4.628E-03	.000	-5.1254E-02	-3.3054E-02
	DW	-5.5840E-03	1.669E-02	.738	-3.8395E-02	2.723E-02
	E2	-.1416*	1.669E-02	.000	-.1744	-.1088
D100	D10	2.637E-02*	4.628E-03	.000	1.727E-02	3.547E-02
	D1000	-1.5780E-02*	4.628E-03	.001	-2.4880E-02	-6.6798E-03
	DW	2.079E-02	1.669E-02	.214	-1.2021E-02	5.360E-02

	E2	-.1152*	1.669E-02	.000	-.1480	-8.2399E-02
D1000	D10	4.215E-02*	4.628E-03	.000	3.305E-02	5.125E-02
	D100	1.578E-02*	4.628E-03	.001	6.680E-03	2.488E-02
	DW	3.657E-02*	1.669E-02	.029	3.759E-03	6.938E-02
	E2	-9.9430E-02*	1.669E-02	.000	-.1322	-6.6619E-02
DW	D10	5.584E-03	1.669E-02	.738	-2.7227E-02	3.840E-02
	D100	-2.0790E-02	1.669E-02	.214	-5.3601E-02	1.202E-02
	D1000	-3.6570E-02*	1.669E-02	.029	-6.9381E-02	-3.7586E-03
	E2	-.1360*	2.314E-02	.000	-.1815	-9.0499E-02
E2	D10	.1416*	1.669E-02	.000	.1088	.1744
	D100	.1152*	1.669E-02	.000	8.240E-02	.1480
	D1000	9.943E-02*	1.669E-02	.000	6.662E-02	.1322
	DW	.1360*	2.314E-02	.000	9.050E-02	.1815

\* The mean difference is significant at the .05 level.

Table 5-40 The uterus weight between regions of Thailand in the dose of 10 mg/kgBw/day.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
North	75	.1218	3.061E-02	3.535E-03	.1148	.1289
North east	20	.1328	2.773E-02	6.200E-03	.1198	.1569
Central	25	.1382	3.543E-02	7.087E-03	.1235	.1715
South	5	.1460	3.445E-02	1.540E-02	.1032	.1888
Total	125	.1278	3.192E-02	2.855E-03	.1222	.1547

Table 5-41 Analysis of variance of uterus weight in 4 regions of Thailand (in the dose of 10 mg/kgBW/day)



	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.494E-03	3	2.498E-03	2.543	.059
Within Groups	.119	121	9.821E-04		
Total	.126	124			

Table 5-42 LSD test of uterus weight between regions of Thailand in the dose of 10 mg/kgBW/day.

(I) code	(J) code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
North	North east	-1.0910E-02	7.887E-03	.169	-2.6524E-02	4.704E-03
	Central	-1.6320E-02*	7.237E-03	.026	-3.0648E-02	-1.9919E-03
	South	-2.4160E-02	1.447E-02	.098	-5.2816E-02	4.496E-03
North east	North	1.091E-02	7.887E-03	.169	-4.7037E-03	2.652E-02
	Central	-5.4100E-03	9.402E-03	.566	-2.4023E-02	1.320E-02
	South	-1.3250E-02	1.567E-02	.399	-4.4271E-02	1.777E-02
Central	North	1.632E-02*	7.237E-03	.026	1.992E-03	3.065E-02
	North east	5.410E-03	9.402E-03	.566	-1.3203E-02	2.402E-02
	South	-7.8400E-03	1.535E-02	.611	-3.8235E-02	2.255E-02
South	North	2.416E-02	1.447E-02	.098	-4.4962E-03	5.282E-02
	North east	1.325E-02	1.567E-02	.399	-1.7771E-02	4.427E-02
	Central	7.840E-03	1.535E-02	.611	-2.2555E-02	3.823E-02

\* The mean difference is significant at the .05 level.

Table 5-43 The uterus weight between regions of Thailand in the dose of 100 mg/kgBw/day.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
North	75	.1509	3.367E-02	3.888E-03	.1431	.1586
North east	20	.1454	2.787E-02	6.232E-03	.1323	.1584
Central	25	.1695	3.481E-02	6.962E-03	.1551	.1838
South	5	.1628	5.132E-02	2.295E-02	9.907E-02	.2265
Total	125	.1542	3.441E-02	3.078E-03	.1481	.1603

Table 5-44 Analysis of variance of uterus weight in 4 regions of Thailand (in the dose of 100 mg/kgBW/day)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.574E-03	3	2.858E-03	2.501	.063
Within Groups	.138	121	1.143E-03		
Total	.147	124			

Table 5-45 LSD test of uterus weight between regions of Thailand in the dose of 100 mg/kgBW/day.

(I) code	(J) code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
North	North east	5.537E-03	8.508E-03	.516	-1.1306E-02	2.238E-02
	Central	-1.8563E-02*	7.807E-03	.019	-3.4020E-02	-3.1071E-03
	South	-1.1913E-02	1.561E-02	.447	-4.2826E-02	1.900E-02
North east	North	-5.5367E-03	8.508E-03	.516	-2.2380E-02	1.131E-02
	Central	-2.4100E-02*	1.014E-02	.019	-4.4178E-02	-4.0218E-03
	South	-1.7450E-02	1.690E-02	.304	-5.0914E-02	1.601E-02
Central	North	1.856E-02*	7.807E-03	.019	3.107E-03	3.402E-02

	North east	2.410E-02*	1.014E-02	.019	4.022E-03	4.418E-02
	South	6.650E-03	1.656E-02	.689	-2.6138E-02	3.944E-02
South	North	1.191E-02	1.561E-02	.447	-1.8999E-02	4.283E-02
	North east	1.745E-02	1.690E-02	.304	-1.6014E-02	5.091E-02
	Central	-6.6500E-03	1.656E-02	.689	-3.9438E-02	2.614E-02

\* The mean difference is significant at the .05 level.

Table 5-46 The uterus weight between regions of Thailand in the dose 1000 mg/kgBw/day.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
North	75	.1681	4.380E-02	5.058E-03	.1581	.1782
North east	20	.1773	3.331E-02	7.448E-03	.1617	.1929
Central	25	.1670	4.567E-02	9.135E-03	.1481	.1858
South	5	.1832	1.809E-02	8.089E-03	.1607	.2057
Total	125	.1700	4.182E-02	3.741E-03	.1626	.1774

Table 5-47 Analysis of variance of uterus weight in 4 regions of Thailand (in the dose 1000 mg/kgBW/day)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.428E-03	3	8.094E-04	.457	.713
Within Groups	.214	121	1.772E-03		
Total	.217	124			

Table 5-48 LSD test of uterus weight between regions of Thailand in the dose of 1000 mg/kgBW/day.

		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval



(I) code	(J) code				Lower Bound	Upper Bound
North	North east	-9.1633E-03	1.059E-02	.389	-3.0138E-02	1.181E-02
	Central	1.177E-03	9.722E-03	.904	-1.8071E-02	2.042E-02
	South	-1.5063E-02	1.944E-02	.440	-5.3558E-02	2.343E-02
North east	North	9.163E-03	1.059E-02	.389	-1.1811E-02	3.014E-02
	Central	1.034E-02	1.263E-02	.415	-1.4663E-02	3.534E-02
	South	-5.9000E-03	2.105E-02	.780	-4.7572E-02	3.577E-02
Central	North	-1.1767E-03	9.722E-03	.904	-2.0424E-02	1.807E-02
	North east	-1.0340E-02	1.263E-02	.415	-3.5343E-02	1.466E-02
	South	-1.6240E-02	2.062E-02	.433	-5.7070E-02	2.459E-02
South	North	1.506E-02	1.944E-02	.440	-2.3431E-02	5.356E-02
	North east	5.900E-03	2.105E-02	.780	-3.5772E-02	4.757E-02
	Central	1.624E-02	2.062E-02	.433	-2.4590E-02	5.707E-02

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