Effects of α -trichosanthin and α -momorcharin on the development of peri-implantation mouse embryos*

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Summary. α -Trichosanthin (0.3 mg/25 g) and α -momorcharin (0.2 mg/25 g) given intraperitoneally to mice on Days 4 and 6 of pregnancy led to an inhibition of implantation. In-vitro study of the effects of these proteins on developing mouse embryos showed that these proteins did not affect the transformation of morulae to blastocysts but further development was impaired: many blastocysts failed to hatch from the zona, the incidence of successful attachment to a plastic substrate decreased and the extent of trophoblastic outgrowth diminished. Inner cell mass development was less affected than was the trophoblast. The in-vivo inhibition of implantation may therefore be a consequence of the deleterious effect of these proteins on the trophoblast.

Introduction

An extract of the root tuber of *Trichosanthes kirilowii* was used in China during the 16th century for inducing menstruation and expulsion of fetal membranes (Anon, 1976a), and an intramuscular injection of 5–10 mg of a crude extract of this plant could induce mid-term abortion at a success rate of 99% (Anon, 1976b). An active component, which is a basic protein of molecular weight 24 000, has been purified from the crude extract and was named α -trichosanthin (Wang, Lin & Zhu, 1976a). This compound is effective in inducing abortion at mid-gestation in mice, rabbits and monkeys (Wang, Ju, Huang & Hsu, 1976b; Chang, Saksena, Lau & Wang, 1979; Saksena, Chang & Lau, 1979; Lau, Saksena & Chang, 1980). α -Trichosanthin has also been used in the treatment of ectopic pregnancy, hydatidiform mole and choriocarcinoma (Anon, 1976a).

However, the usefulness of α -trichosanthin for termination of early pregnancy (during the first trimester in man or during the implantation stage in animals) is uncertain. When α -trichosanthin was given to pregnant mice (50–200 μ g) intraperitoneally on Days 1–4, no disruption of gestation was found (Chang *et al.*, 1979). However, when α -trichosanthin was administered together with other traditional Chinese medicine, or with reserpine and testosterone, total inhibition of early pregnancy was found in man, mouse, rat and rabbit (Lau, Lau, Lau, Ming & Sun, 1981; Jin & Ho, 1981). In another study, α -trichosanthin was given to pregnant mice as two successive doses (250 μ g each) on Days 4 and 5 and this resulted in complete inhibition of pregnancy (Zhou, Li, Shu, Bao & Chu, 1982). Clearly, the dosage, the stage of pregnancy when the drug is administered and the regimen of drug treatment are important factors in determining the abortifacient effect of the protein.

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In the present study, we have investigated the effects of α -trichosanthin and α -momorcharin, which is isolated from the seeds of *Momordica charantia*, on pregnancy in the mouse by giving the proteins to the pregnant animal at stages when embryos are implanting in the uterus. We have observed also the in-vitro development of the peri-implantation embryos in the presence of α -trichosanthin and α -momorcharin to study effects of the proteins on the developmental potential of specific cell populations in the embryo.

Materials and Methods

Random bred ICR female mice were caged with males overnight. The presence of vaginal plugs was checked the next morning (Day 1 of pregnancy). α -Trichosanthin was purified and prepared as described by Yeung & Li (1983). α -Momorcharin (a basic glycoprotein, M_r 31 000) was purified by subjecting an extract of the seeds of *Momordica charantia* to acetone fractionation, and this fraction was further purified on CM Sepharose CL 6B and Sephadex G100 (fine) columns (Pharmacia, Uppsala, Sweden). The final preparation was shown to be homogeneous by SDS-polyacrylamide gel electrophoresis and immunoelectrophoresis.

Effects on pregnancy. Pregnant females were given a single intraperitoneal injection of either protein on Days 4, 6 or 8 of pregnancy. Different doses were used (see Text-fig. 1). Animals were autopsied on Day 11 of pregnancy. The embryos were examined for normal morphology and the number of viable embryos was scored. The abortifacient effect of the proteins was expressed as the percentage of non-pregnancy per treatment. This percentage was corrected for an intrinsic non-pregnancy rate which was estimated from the incidence of non-pregnant mice in the control groups (8/48, 3 expts).

Effects on embryonic development in vitro. Preimplantation embryos were collected from mice on Day 4 of pregnancy by flushing the uterus with phosphate-buffered saline. The embryos were then washed with pre-warmed culture medium. The culture medium was prepared by mixing Dulbecco's modified Eagles Medium (DMEM; Gibco, Grand Island, N.Y., U.S.A.) with 10% fetal calf serum (of selected batches; Gibco) to which penicillin (10^5 U/l) and streptomycin (50 mg/l) were added. In each culture experiment, an assortment of compacted morulae to fully expanded blastocysts was used. The embryos were cultured in groups of 10–15 in 1 ml medium at 37°C in an atmosphere of 5% CO₂ in air and on a Falcon Multiwell tissue culture plate.

The effects of α -trichosanthin and α -momorcharin on the hatching of blastocysts were studied by exposing zona-covered embryos to the proteins in culture. Various doses were added to the medium in small volumes of phosphate-buffered saline. The cultures were monitored at intervals of 12 h under an inverted microscope and complete herniation of the blastocyst from the zona was taken as a successful hatching. These embryos were cultured for 5–6 days to observe their subsequent development.

Zona-free embryos were used in the studies on the effect of the proteins on trophoblast attachment and outgrowth. The zona was removed enzymically by treating the embryo with pronase (0.5% in phosphate-buffered saline supplemented with 1% polyvinylpyrrolidone (Sigma, St Louis, Missouri, U.S.A.)) for 10 min. The embryos were then washed with the culture medium and incubated for several hours in fresh medium at 37°C before further experimental treatment. At regular intervals, the culture plate was shaken gently and a successful attachment was scored when the motion of the plate failed to dislodge the embryo. To study the effect of the proteins on trophoblast differentiation and inner cell mass development, zona-free embryos were cultured to the stage of initial spreading of the trophoblast (normally 48 h after culture) and then the proteins were added. The cultures were followed for another 48 h after treatment. The outgrowth was recorded with a NIKON inverted microscope using phase optics and its area was measured on the print by using a planimeter. The formation of both a proper inner cell mass and an egg cylinder was also scored as an index of inner cell mass development. Representative embryos from each

treatment group were processed for histology with fixation in half-strength Karnovsky fixative and embedding in Spurr plastics. Serial 2 μ m sections were stained with 1% toluidine blue.

Results

Effects on early pregnancy (see Text-fig. 1)

α -Trichosanthin given intraperitoneally to mice on Day 4 of pregnancy resulted in an inhibition of pregnancy in 80% of the mice, and on Day 6, to a lesser extent of 35%. Severe bleeding from the vagina and abortion of conceptuses were observed in mice treated on Day 8. α -Momorcharin given to pregnant mice on Days 4 or 6 of pregnancy resulted in an inhibition of pregnancy in 57–64% of mice, but had no effect when given on Day 8 of pregnancy. This suggests that the abortifacient action of the proteins varies with the stages of implantation. The response of pregnant mice to either abortifacient followed an all-or-none pattern, i.e. the entire litter was aborted or survived the treatment. The average number of conceptuses per litter, which was about 12.9 (s.e.m. = 0.2, n = 63), was the same amongst the controls and the survivors in the treatment groups. The gross morphology of surviving embryos was normal and there were no signs of retarded development.

Text-fig. 1. Effects of α -trichosanthin and α -momorcharin on early pregnancy (Days 1–4) of mice weighing 27–34 g. Data were pooled from 3 experiments. ★ Significantly different from the control at $P < 0.01$ (Fisher's exact test applied to the raw data). I = control (vehicle only), II = α -trichosanthin (0.3 mg/25 g body wt), III = α -trichosanthin (0.1 mg/25 g body wt), IV = α -momorcharin (0.2 mg/25 g body wt). N = no. of mice.

Effects on hatching in vitro

In our culture, 96–100% of the morulae developed to blastocysts after 18–24 h in the control groups and in those treated with α -trichosanthin or α -momorcharin. In the control group, about 50% of the embryos had hatched from the zona after 48 h *in vitro* and over 70% by 72 h *in vitro*. The

hatched embryos then attached to the culture plate, the trophoblasts formed outgrowths and the inner cell mass developed into an early egg cylinder (Pl. 1, Figs 4 & 5). When 0.15 µg/ml or more of α-trichosanthin or α-momorcharin was present in the culture medium, significantly fewer embryos managed to hatch from the zona (Table 1). The unhatched embryos showed obvious signs of cell necrosis and the blastocoelic cavity rapidly collapsed. However, the embryos that did hatch successfully developed normally through the attachment and outgrowth stages in a fashion indistinguishable from untreated embryos (Table 1).

Table 1. Effects of α-trichosanthin and α-momorcharin on in-vitro development of zona-covered embryos

Treatment	No. of embryos (2 expts)	No. (%) of embryos hatched at:			Development subsequent to hatching			
		24 h	48 h	72 h	48 h		72 h	
					Attached	Outgrowth	Attached	Outgrowth
Control	37	2 (5)	19 (51)	26 (70)	20 (77)	6 (23)	26 (100)	24 (92)
α-Trichosanthin (µg/ml)								
0.015	22	0	9 (41)	14 (64)	3 (21)	0 (0)	14 (100)	14 (100)
0.15	30	2 (7)	7 (23)*	12 (40)*	10 (83)	0 (0)	12 (100)	10 (83)
0.75	19	0	3 (16)*	4 (21)*	3 (75)	2 (50)	4 (100)	4 (100)
1.50	19	0	0 (0)	0 (0)	0	0	0	0
Control	49 (2)	0	26 (53)	35 (71)	20 (57)	6 (17)	35 (100)	33 (94)
α-Momorcharin (µg/ml)								
0.015	22 (2)	0	12 (54)	16 (73)	8 (50)	0	16 (100)	15 (94)
0.15	22 (2)	0	5 (23)*	12 (54)*	6 (50)	0	12 (100)	8 (67)
0.75	11 (1)	0	0	1 (9)*	0	0	1 (100)	1 (100)
1.50	23 (2)	0	0	0	0	0	0	0

Values in parentheses are percentages.

* Significantly different from control values at $P < 0.01$ (χ^2). Zero values were not tested.

Effects on attachment in vitro

The embryos used in this study were stripped of their zonae so that the initial hatching stage was bypassed. In the control group, about 20% of the zona-free embryos attached to the culture plate after 24 h *in vitro* and almost all of them had attached after 72 h *in vitro* (Text-fig. 2). In the presence of ≥ 1.5 µg/ml or more of α-trichosanthin and α-momorcharin in the culture medium, significantly fewer ($P < 0.01$ by χ^2 test) embryos attached to the culture plate after 48 and 72 h in culture (Text-fig. 2).

The outgrowth of trophoblast from the attached embryos in the control and the treatment groups was analysed by measuring the area of the outgrowth at 72 h and 92 h *in vitro* (Table 2). The embryos treated with α-trichosanthin and α-momorcharin formed smaller outgrowths than did the untreated embryos at both times (Pl. 1, Figs 1, 2 & 3), and the effect was greater with the higher concentrations of the proteins (Table 2).

Effects on trophoblast outgrowth

Zona-free embryos were cultured to the stage of early outgrowth of trophoblast and were then exposed to various concentrations of proteins. During the first 24 h, only embryos treated with the

(Facing p. 600)

Text-fig. 2. Effects of (a) α -trichosanthin and (b) α -momorcharin on the attachment of zona-free embryos. Concentrations of the protein in the culture medium were $\mu\text{g/ml}$. PBS only was added in the control. The no. of embryos studied is given in parentheses.

highest concentration (30 $\mu\text{g/ml}$) of the proteins showed a retardation of outgrowth (Text-fig. 3). In the next 24 h of treatment, all treated embryos, except those in groups treated with lowest concentrations of the proteins, showed a reduction in outgrowth expansion (Text-fig. 3). Very often, isolated trophoblast cells were found outside the main outgrowth or rounded up on top of the

Text-fig. 3. Effects of α -trichosanthin and α -momorcharin on trophoblast outgrowth of mouse embryos *in vitro*. Values are the mean $\pm$ s.e.m. of the no. of outgrowths analysed (n) of the differences in log area of outgrowth between 0 h and 24 or 48 h. *Significant difference at $P < 0.01$ (Student's unrelated t test). C = control; I = 0.15 μg protein/ml, II = 1.5 μg protein/ml, III = 15.0 μg protein/ml, IV = 30.0 μg protein/ml.

Table 2. The outgrowth of zona-free embryos treated with α -trichosanthin and α -momorcharin before attachment *in vitro*

Treatment	No. of outgrowths analysed	Area (range) of outgrowth (10 ⁴ μ m ²)		Mean increase in log area	Within group increase in area Significance (paired <i>t</i> test)
		72 h	96 h		
Control	13	6.3 (2.4–11.8)	8.7 (6.1–13.0)	0.1924 $\pm$ 0.0394	<i>P</i> < 0.001
α -Trichosanthin (μ g/ml)					
0.15	13	5.8 (3.1–9.5)	7.6 (5.1–10.8)	0.1546 $\pm$ 0.0234	<i>P</i> < 0.001
0.75	7	3.4 (1.6–5.0)	3.5 (1.6–5.0)***	0.0237 $\pm$ 0.0134†	—
1.50	7	1.7 (0.4–4.2)**	2.1 (0.6–4.9)***	0.1229 $\pm$ 0.0450	<i>P</i> < 0.01
7.50	7	0.95 (0.5–2.1)***	1.0 (0.4–1.8)***	0.0147 $\pm$ 0.0628‡	—
α -Momorcharin (μ g/ml)					
0.15	12	4.4 (2.0–6.5)	4.7 (2.0–7.5)***	0.0239 $\pm$ 0.0386†	—
0.75	8	2.0 (0.9–4.2)**	2.4 (0.9–4.9)***	0.0987 $\pm$ 0.0131	<i>P</i> < 0.01
1.50	5	2.1 (0.4–4.6)*	2.4 (1.1–5.0)***	0.1206 $\pm$ 0.0797	—
7.50	8	0.91 (0.4–1.1)***	1.1 (0.4–2.0)***	0.0863 $\pm$ 0.0708	—

Significantly different from control value: * *P* < 0.025, ** *P* < 0.01, *** *P* < 0.001 (K-S two-sample test for non-parametric variables).

Significantly different from control value: † *P* < 0.025, ‡ *P* < 0.01 (Student's unrelated *t* test).

outgrowth. An estimation of the numbers of trophoblast cells from photographs of the outgrowths revealed that the outgrowths of treated embryos contained about 40–70% of the number of trophoblast cells normally found in the controls.

Effects on inner cell mass differentiation

Over 90% of the untreated embryos and treated embryos developed a proper inner cell mass with embryonic ectoderm and endoderm (Pl. 1, Figs 4, 5, 6 & 7). However, vacuolation and possibly

PLATE 1

Fig. 1. Control (untreated) embryos after 72 h *in vitro*, showing extensive outgrowth of trophoblast and development of inner cell mass. Bar = 100 μ m.

Fig. 2. Embryos after 72 h of culture in the presence of 1.5 μ g α -trichosanthin/ml, showing a reduced outgrowth of trophoblast. Bar = 100 μ m.

Fig. 3. Embryos after 72 h of culture in the presence of 1.5 μ g α -momorcharin/ml, showing reduction of trophoblastic outgrowth. Bar = 100 μ m.

Fig. 4. Control (untreated) zona-free embryo after 96 h of culture, showing the differentiation of trophoblast (arrow), the embryonic ectoderm (E) and the vacuolated endoderm (N). The section was taken parallel to the surface of the culture plate. Bar = 50 μ m.

Fig. 5. Control (untreated) zona-free embryo after 96 h of culture, showing the differentiation of trophoblast (arrow), and the formation of an early egg cylinder (EG). Bar = 50 μ m.

Fig. 6. Zona-free embryo cultured for 96 h in 1.5 μ g α -trichosanthin/ml, showing poor development of trophoblast (arrow) and the presence of necrotic cells (*) in the egg cylinder. Bar = 50 μ m.

Fig. 7. Zona-free embryo cultured for 96 h in 1.5 μ g α -momorcharin/ml, showing poor development of trophoblast (arrow) and the early egg cylinder (EG). Degenerative changes of cells (*) in the embryonic ectoderm were observed. Bar = 50 μ m.

cell necrosis were evident to some extent in the embryonic ectoderm of protein-treated embryos (Pl. 1, Figs 6 & 7). The treated embryos generally formed smaller egg cylinders than did the controls, suggesting that inner cell mass development had been retarded concomitantly with a deficiency in trophoblast differentiation.

Discussion

Treatment of pregnant mice with α -trichosanthin at the stage of embryonic implantation very effectively inhibited pregnancy. This agrees with the observations made by Zhou *et al.* (1982) but is in contrast to the results of Chang *et al.* (1979). The reasons for this apparent discrepancy of results are not known, but factors such as the quantity of protein administered, the potency and purity of preparations and the treatment regimen may be involved. α -Momorcharin was also abortifacient when it was given to pregnant mice at the stage of implantation, but became ineffective shortly afterwards when embryos have fully implanted.

Both α -trichosanthin and α -momorcharin induce abortion when they are administered to pregnant mice at Day 9–14 (unpublished observation). This phenomenon of mid-term abortion, which is also seen in women and in other animals, has been attributed to the selective destruction of placental villi (Anon, 1976b). Various studies on the tissue localization of α -trichosanthin, on the ultrastructural changes in the placenta and on the functional competency of trophoblast cells (as indicated by hCG production) have produced results which suggest that the syncytiotrophoblast is always preferentially affected (Hsu, Jiang, Chen & Huang, 1976; Kao & Chou, 1978; Tso, Liu & Liu, 1976; Tso, Wang & Wang, 1978; Wang *et al.*, 1976b; Wang, Hsu & Chiang, 1978; Young, 1978). α -Trichosanthin has also been shown to impair the secretion of hCG and to cause degenerative changes in human syncytiotrophoblast maintained *in vitro* (Xiong *et al.*, 1976). The mechanisms of action of these proteins in preventing early pregnancy are less clear. Several factors, such as a disturbance of the tonic condition of the uterus, an inhibition of normal decidual formation and the damage of cells in the implanting blastocysts by the protein, have been implicated (Zhou *et al.*, 1982; Tseng, Teng, Wu & Chou, 1979). In the present study, by using an *in vitro* culture system, we have found that both proteins retarded several important steps of embryonic development *in vitro*. Treatment of zona-covered embryos led to impaired hatching, while treatment of zona-free embryos diminished the incidence of trophoblast attachment to the culture substrate and reduced the amount of trophoblast outgrowth. Inner cell mass differentiation was also disturbed but apparently to a lesser extent than were the trophoblast cells. Egg cylinders were formed in treated embryos but there was some histological evidence of cellular damage in the embryonic ectoderm. It seems likely, therefore, that the abortifacient effect of α -trichosanthin and α -momorcharin is partly mediated through a mechanism whereby the trophoblast cells are rendered incapable of normal differentiation, leading to unsuccessful implantation. It may seem paradoxical that inhibition of pregnancy *in vivo* is an all-or-none effect whereas inhibition of blastocyst development *in vitro* is a continuously variable response. There is at present no information on the amount of protein that could reach the embryos *in vivo* but it could vary in individual animals. The total inhibitory effect *in vivo* is mimicked to some extent by the complete arrest of blastocyst development in cultures with a high concentration of proteins. However, the viability of protein-treated blastocysts should be tested by transferring them to a pseudopregnant uterus.

α -Trichosanthin has been shown to exert a suppressive effect on human choriocarcinoma which takes its origin from malignant transformation of trophoblast in hydatiform moles (Anon, 1976b). The vulnerability of trophoblast to trichosanthin may be related to the inherent property of the cells to undergo malignant transformation and to become invasive and pinocytotic when they come into contact with other tissues (Sharpey-Shafer & Ockleford, 1978). A number of proteinaceous plant toxins are known to kill normal and tumour cells *in vitro* (Shoham, Inbar & Sachs, 1970; Lin,

Kau, Tseng, Chan & Tung, 1970; Inbar, Ben-Bassat & Sachs, 1972; Lin, Hou & Chen, 1978). These plant toxins first bind to cell surface receptors and then become internalized. The cytotoxic effect is the result of suppression of protein synthesis by the internalized toxin molecules (Olsnes, Refsnes & Phil, 1974).

References

- Anon (1976a) Shanghai Institute of Experimental Biology. Studies on the mechanisms of abortion induced by trichosanthin. *Scientia sin.* **19**, 811–827.
- Anon (1976b) 200 cases of mid-term abortion by trichosanthin. *J. Chin. Med.* **56**, 215–221 (in Chinese).
- Chang, M.C., Saksena, S.K., Lau, I.F. & Wang, Y.H. (1979) Induction of midterm abortion by trichosanthin in laboratory animals. *Contraception* **19**, 175–184.
- Hsu, K.J., Jiang, W.S., Chen, Y.F. & Huang, M.B. (1976) Studies on the site of induced abortion by trichosanthin. *Acta zool. sin.* **22**, 149–155 (in Chinese).
- Inbar, M., Ben-Bassat, H. & Sachs, L. (1972) Inhibition of ascites tumor development by concanavalin A. *Int. J. Cancer* **9**, 143–149.
- Jin, Y.C. & Ho, C.C. (1981) Trichosanthin, prostaglandin and traditional Chinese medicines for the termination of early pregnancy. *Reproduction and Contraception* **1**, 19–23 (in Chinese).
- Kao, H. & Chou, S.G. (1978) Studies on the localisation of trichosanthin *in vivo* by autoradiography. *Acta Biol. exp. sin.* **11**, 253–259 (in Chinese).
- Lau, I.F., Saksena, S.K. & Chang, M.C. (1980) Further studies on the trichosanthin induced termination of pregnancy. *Contraception* **21**, 77–86.
- Lau, K.W., Lau, T., Lau, F.Y., Ming, T.Y. & Sun, P.L. (1981) Inhibition of early pregnancy by combined trichosanthin in laboratory animals. *Reproduction and Contraception* **1**, 20–24 (in Chinese).
- Lin, J.Y., Kau, W.Y., Tseng, K.Y., Chen, C.C. & Tung, T.C. (1970) Effect of crystalline abrin in the biosynthesis of protein, RNA and DNA in experimental tumors. *Cancer Res.* **30**, 2431–2433.
- Lin, J.Y., Hou, M.J. & Chen, Y.C. (1978) Isolation of toxic and nontoxic lectins from the bitter pear melon *Momordica charantia* L. *Toxicon* **16**, 653–660.
- Olsnes, S., Refsnes, K. & Phil, A. (1974) Mechanism of actions of the toxic lectins abrin and ricin. *Nature, Lond.* **249**, 627–631.
- Saksena, S.K., Chang, M.C. & Lau, I.F. (1979) Termination of pregnancy in rabbit and mouse by trichosanthin. *Contraception* **20**, 367–376.
- Sharpey-Shafer, J.M. & Ockleford, C.D. (1978) Uptake of protein by cultured human trophoblast cells. *Cell Biol. Int. Reports* **2**, 579–589.
- Shoham, J., Inbar, M. & Sachs, L. (1970) Differential toxicity on normal and transformed cells *in vitro* and inhibition of tumour development *in vivo* by concanavalin A. *Nature, Lond.* **227**, 1244–1246.
- Tseng, M.P., Teng, M.Y., Wu, H.L. & Chou, M.Y. (1979) Electron microscopic observations on the effect of trichosanthin to blastocyst. *Acta Biol. exp. sin.* **12**, 267–275.
- Tso, J.K., Liu, S.F. & Liu, L.Y. (1976) Serum HCG levels of women during and after abortion induction by trichosanthin. *Acta zool. sin.* **22**, 166–171.
- Tso, J.K., Wang, Y.P. & Wang, M.P. (1978) Radioimmunoassay of trichosanthin. *Acta Biol. exp. sin.* **11**, 277–290.
- Wang, Y.T., Lin, J.F. & Zhu, L.X. (1976a) Preliminary studies on purification and characterisation of an abortifacient plant protein, trichosanthin. *Acta zool. sin.* **22**, 137–143 (in Chinese).
- Wang, Y.T., Ju, R.M., Huang, J.H. & Hsu, K.C. (1976b) Investigations on the injurious effects of the abortifacient-trichosanthin to monkey placental villi. *Acta zool. sin.* **22**, 156–165 (in Chinese).
- Wang, Y.T., Hsu, K.C. & Chiang, W.S. (1978) The ultrastructural observations on the injurious effects of the abortifacient trichosanthin on monkey trophoblast. *Acta Biol. exp. sin.* **11**, 236–270 (in Chinese).
- Xiong, Y.Z., Pan, P., Xu, S.Y., Zhang, J., Wang, Y.H., Zuo, J.K. & Gu, Z. (1976) Study on the effects of trichosanthin on human trophoblast cell *in vitro*. *Acta zool. sin.* **22**, 172–179 (in Chinese).
- Yeung, H.W. & Li, W.W. (1983) Bioactive peptide and protein from Chinese medicinal materials I. α -trichosanthin: a new abortifacient protein from the Chinese drug, Wangau (*Trichosanthes cucumerides*). *Int. J. Peptide Protein Res.* (in press).
- Young, P.Y. (1978) Placentopathology of trichosanthin induced mid-term abortion. *J. Chin. Med.* **3**, 160–162 (in Chinese).
- Zhou, M.H., Li, Q., Shu, H.D., Bao, Y.M. & Chu, Y.R. (1982) Pharmacological study of the effect of Radix Trichosanthis on terminating early pregnancy. *Acta pharm. sin.* **17**, 176–181 (in Chinese).

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