



浙江中醫藥大學  
ZHEJIANG CHINESE MEDICAL UNIVERSITY



# Evaluation of in vitro embryo-toxicity tests for Chinese herbal medicines

**Lucia Lu LI, M.B.B.S, Ph.D.**

Zhejiang Chinese Medical University, China

**Horst Spielmann**

FU Berlin, Germany

Freie Universität



Berlin

# Evaluation of *in vitro* embryotoxicity tests for Chinese herbal medicines

Lu Li<sup>a,b,c</sup>, Ling Yin Tang<sup>b</sup>, Bo Liang<sup>b</sup>, Rongyun Wang<sup>d</sup>, Qiuhua Sun<sup>d</sup>, Clara Bik San Lau<sup>c</sup>,  
Ping Chung Leung<sup>c</sup>, Ellen Fritsche<sup>e</sup>, Manfred Liebsch<sup>f</sup>, Andrea E.M. Seiler Wulczyn<sup>f</sup>,  
Horst Spielmann<sup>g</sup>, Chi C. Wang<sup>a,b,h,i,\*</sup>

<sup>a</sup> College of Basic Medical Sciences, Zhejiang Chinese Medical University, Hangzhou, China

<sup>b</sup> Department of Obstetrics & Gynaecology, The Chinese University of Hong Kong, Shatin, Hong Kong



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Reproductive Toxicology

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**The validated embryonic stem cell test to predict  
embryotoxicity *in vitro***

**Andrea E M Seiler<sup>1</sup> & Horst Spielmann<sup>2</sup>**

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# Background



# Traditional Chinese Medicine

- Widely applied with long history
- Promote both mothers' & fetuses' health
- Effective in many pregnancy disorders
- Few embryotoxic effects
- Lacking scientific evidence of its claimed applications

# Therapeutic Approaches

- Chinese medicines
- Acupuncture
- Food therapy
- Tai Chi exercise
- Qi Gong
- Cupping
- Tui Na (Physical therapy)
- Die Da
- Gua Sha

# Chinese medicines

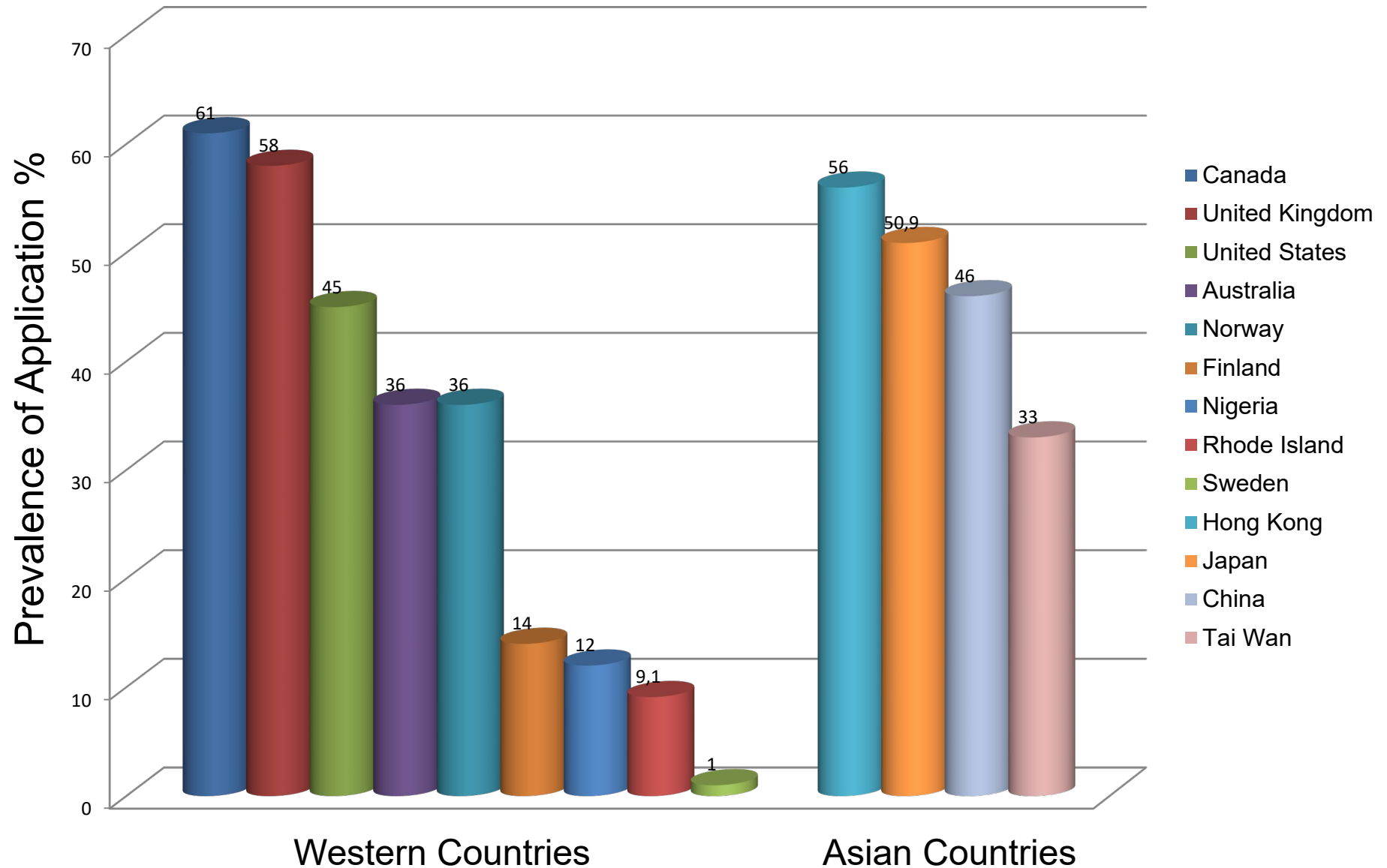
## Origins

- Plant original (85%)  
= Chinese herbal medicines (CHMs)
- Animal original (10%)
- Mineral original (5%)

## Chinese Pharmacopeia

- 6,000 CHMs
- 250 CHMs commonly for pregnancy
- 31 CHMs forbidden during pregnancy

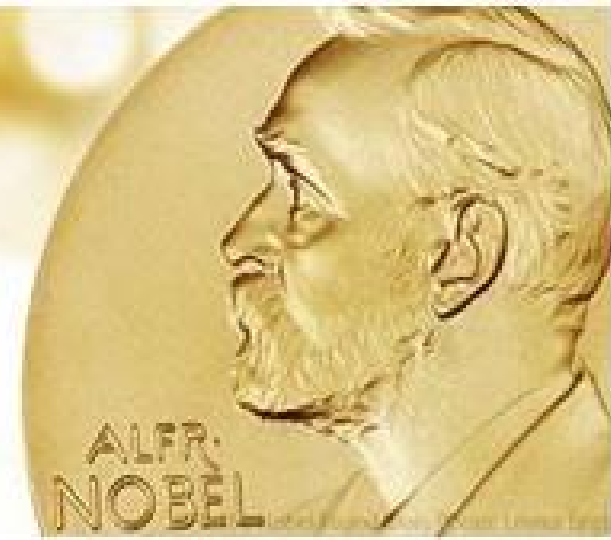
# Prevalence during pregnancy



*"For the greatest benefit to mankind"*  
*Alfred Nobel*

2015 NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE

**William C. Campbell**  
**Satoshi Ōmura**  
**Youyou Tu**





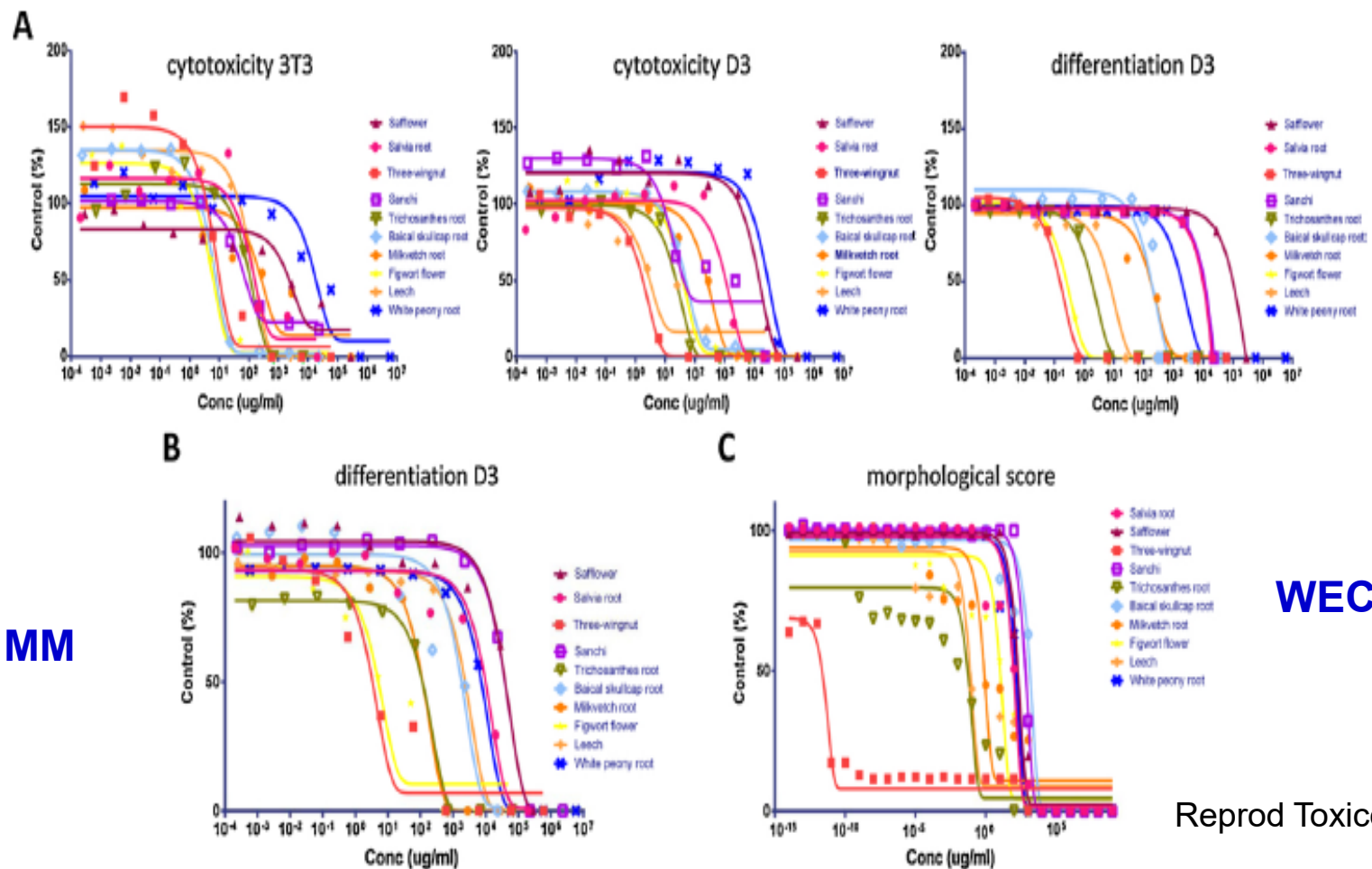
# Aim

- CHMs have been widely used during pregnancy,
- but feto-embryo safety tests are lacking.
- Here we evaluated *in vitro embryotoxicity tests (IVTs)* as alternative methods in assessing developmental toxicity of CHMs,
- to report the importance of validated in vitro toxicity tests for the safety testing of CHMs.

# Previous Work - Report

- Ten CHMs were selected and classified as strongly, weakly and non-embryotoxic.
- All test CHMs were authentication qualified.
- Three well validated IVTs and prediction models (PMs) were compared.
  - - embryonic stem cell test (EST),
  - - micromass (MM)
  - - whole embryo culture (WEC)

EST



Reprod Toxicol 2019


Fig. 1. Dose response curves of Chinese herbal medicines in *in vitro* embryotoxicity tests. A. Cytotoxicity and differentiation of 3T3 and D3 cells in embryonic stem cell test. B. Differentiation of dissociated limb buds in micromass test. C. Morphological score of embryo development in whole mouse embryo culture test. Mean values and representative regression lines from 3 replicated samples in 3 independent experiments are presented.

**All strongly embryotoxic CHMs were predicted by MM and WEC PM2. While all weakly embryotoxic CHMs were predicted by MM and WEC PM1. All non-embryotoxic CHMs were classified by EST, MM, but over-classified as weakly embryotoxic by WEC PM1.**

# Our findings

- Overall predictivity, precision and accuracy of WEC determined by PM2 were better than EST and MM tests.
- Compared with validated chemicals, performance of IVTs for CHMs was comparable.
- So IVTs are adequate to identify and exclude embryotoxic potential of CHMs in this training set.

# Chinese herbal medicines of PHF

Pharmaceutical Name	English Name	Chinese Name	CHM images	Yield (%)	Clinical Dose	LD <sub>50</sub> (g/kg) (mice)
Flos Ionicerae	Honeysuckle Flower	Jin Yin Hua (JYH)		34.2%	6-15g (100-250mg/kg)	67.6~81.7
Cortex moutan	Tree Peony Bark	Mu Dan Pi (MDP)		23.4%	6-9g (100-150mg/kg)	3.4
Cortex phellodendri	Amur Corktree Bark	Huang Bai (HB)		13.7%	3-12g (50-200mg/kg)	2.7
Herba menthae	Common Mint	Bo He (BH)		28.6%	3-6g (50-100mg/kg)	3.3
Rhizoma atratylodis	Swordlike Atractylodes Rhizome	Cang Zhu (CZ)		34.0%	3-9g (50-150mg/kg)	≥ 5.0

**Pentaherbs Formulation (PHF): 2:2:1:2:2, and clinical effects have been proved.**



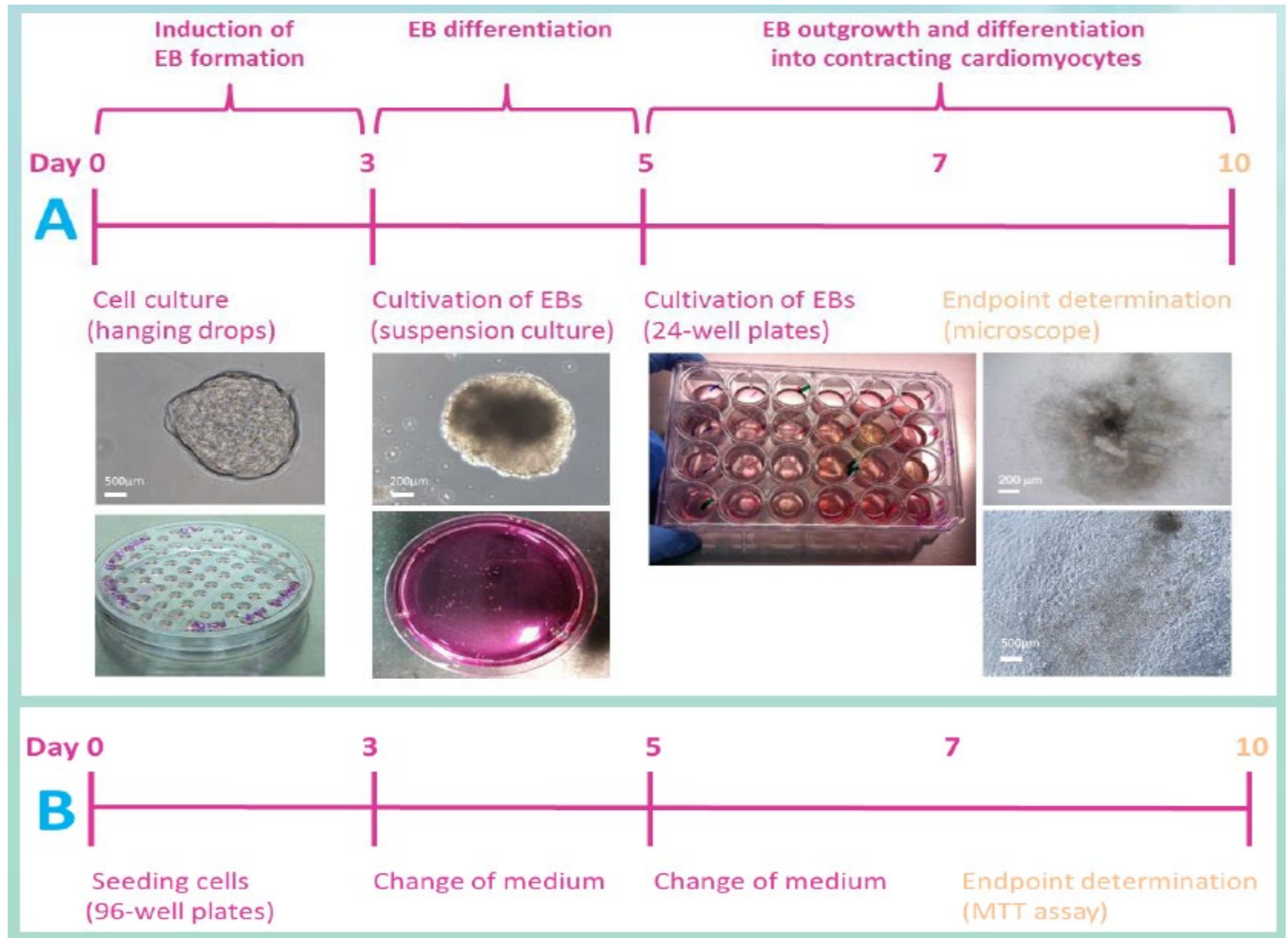
# Objectives

- To **evaluate** and **predict** the **embryotoxicity** potentials of a **CHM** formula used for the treatment of atopic dermatitis **by** Embryonic Stem Cell Test (**EST**)

## Methods

- EST - Differentiation assay (embryonic stem cells)
- EST - Cytotoxicity assay (embryonic stem cells & fibroblast)
- Skin irritation test – cytotoxicity assay (EPI-200 skin tissues)

# Overview of EST Protocol





# Summary I - EST results

## Classification:

(A Seiler, nature protocols, 2011)

Non-embryotoxic

If I>II and I>III

Weakly embryotoxic

If II>I and II>III

Strongly embryotoxic

If III>I and III>II

	JYH	MDP	HB	BH (Mint)	CZ	PHF formula
Functions Results	I>II, I>III	I>II, I>III	II>I, II>III	I>II, I>III	I>II, I>III	I>II, I>III
Classification (EST)	Non-embryotoxic	Non-embryotoxic	Weakly-embryotoxic	Non-embryotoxic	Non-embryotoxic	Non-embryotoxic
N number	3~4	3~4	3~4	3~4	3~4	3~4

Pharmaceutical	English	Chinese	Chinese	IC50 3T3	IC50 D3	ID50 D3	Functions			EST
Name	Name	PinYin	Name	µg/ml	µg/ml	µg/ml	I	II	III	Prediction
Flos Ionicerae	Honeysuckle Flower	Jin Ying Hua	金银花	375,4	1151	360,9	10.0496685	9.78446441	-8.811138206	non
Herba menthae	Common Mint	Bo He	薄荷	122,6	444,8	252,7	11,5672772	9,25628838	-9,607299511	non
Cortex moutan	Tree Peony Bark	Mu Dan Pi	牡丹皮	113,8	316,7	342,8	15,9099555	10,7164418	-10,74671549	non
Cortex phellodendri	Amur Corktree Bark	Huang Bai	黄柏	399,4	3,119	1,37	-3,8623964	1,80279936	-2,448830745	WEAKLY
Rhizoma atratylodis	Swordlike Atractylodes Rhizome	Cang Zhu	苍术	400.5	1198	467,5	11.3703204	10.3466115	-9.156898735	non
	Formula (PHF)			231,2	258,5	347,1	9,40031001	8,56197037	-8,349369097	non



# Conclusion

## mEST in vitro embryotoxicity test

- **Penta Herbs Formulation (PHF) should not be used during pregnancy**
- The potentially embryotoxic **Amur Corktree Bark** to be eliminated from HPF and replaced by another CHM

# First internet information platform

## Safety of Chinese Herbal Medicines for Pregnancy

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Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong

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In Asia countries, many pregnant women avoid Western medicines but opt for Chinese medicines to prevent its adverse effects on their fetuses. However, it is not very clear how safe the Chinese medicines are being used during pregnancy and if there are any toxicity or side-effects to both the mothers and babies. Here, we provide general and in-depth information regarding the safety concerns of Chinese herbal medicines during pregnancy.



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# Hangzhou, China



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