Coptis Rhizome and Phellodendron Bark Extracts and Berberine Inhibit the Development of Mouse Embryos

Yukio Tsunoda and Yoko Kato*

Laboratory of Animal Reproduction, College of Agriculture, Kinki University, Nara 631-8505, Japan

Abstract: *After screening 269 crude drugs for their ability to inhibit the development of mouse zygotes, we found Coptis rhizome and Phellodendron bark to have inhibitory effects. We examined the effects of both extracts and of berberine, a major component of these plants, on in vitro development of zygotes and on full-term fetal development in the mouse. Mouse zygotes were cultured in medium containing water-soluble extracts of Coptis rhizome or Phellodendron bark, or berberine at various concentrations for 5 days and the potential of zygotes to develop to blastocysts was examined. In addition, superovulated mice were intramuscularly injected with berberine and mated, and examined for the in vivo development of fertilized eggs to blastocysts and full-term fetuses. In vitro development of zygotes to blastocysts was almost completely inhibited when they were cultured in medium containing more than 0.1 μg/ml Coptis rhizome, 10 μg/ml Phellodendron bark, or 0.01 μg/ml berberine chloride or berberine sulfate. When superovulated and mated females received 100 μg berberine chloride once a day for 2 to 14 days, the proportions of recovered blastocysts and full-term fetuses were significantly decreased. The present study indicates the potential use of berberine as a contraceptive for animals.*

Key words: *Coptis rhizome, Phellodendron bark, Berberine, Antifertility*

Introduction

Assisted reproductive technologies such as *in vitro* fertilization, intracytoplasmic sperm injection, freezing of embryos and gametes are now widely used in animal husbandry, to rescue endangered species, as well as in human infertility therapy. Somatic cell nuclear transfer technology has also been developed to address these needs [1]. Fertility reduction and contraception in female animals are also important for avoiding unplanned reproduction in companion animals, wild animals and animals in zoos. Although effective contraceptive pills to control human fertility have been developed [2], practical non-invasive methods to inhibit fertility in female animals have not yet been established.

A few natural products are known to have an anti-fertility effect on animals. One example is gossypol, a natural component of cottonseed, which inhibits fertility [3]. Gossypol is a toxic factor indigenous to the cotton plant genus. *In vitro* treatment of gametes with gossypol or *in vivo* administration of gossypol disrupts estrous cycles, pregnancy, early embryonic development, sperm viability and sperm count [4, 5]. Gossypol administration in feed, especially to non-ruminant and immature ruminant animals, however, also produces toxic effects such as labored breathing, dyspnea, depressed growth rate, and anorexia [4].

In our preliminary studies, we examined the *in vitro* development of mouse zygotes treated with 269 crude drugs, as listed in Table 1, at a concentration of 10 μg/ml. We found that 2 of the 269 crude drugs in the list, Coptis rhizome and Phellodendron bark, had inhibitory effects on the *in vitro* development of mouse zygotes to blastocysts. The roots of Coptis rhizome and Phellodendron bark are well known and widely used in traditional oriental herbal medicine. Coptis rhizome and Phellodendron bark contain berberine, which has anti-malarial, anti-diarrheal, anti-inflammatory, and anti-microorganism effects, as well as inhibitory effect on morphine-induced locomotor sensitization [6, 7].

In the present study, we demonstrated for the first

Received: July 26, 2010
Accepted: November 30, 2010
*To whom correspondence should be addressed.
e-mail: tsunoda@nara.kindai.ac.jp

Table 1. List of crude drugs examined

Agaricus subrufescens	Astragalus root	Platycodon root	Epicatechin gallate	Processed aconite root	Japanese angelica bark	Low striped bamboon
Lycium fluit	Perilla herb	Cyperus rhizome	Yutan-gan	Upland white	Japanese yew	Sweet hydrangea leaf
Ginseng	Artremisia capillaris flower(1)	Mulberry bark	Zuishi	Rangoon creeper fruit	Rose fruit	Talc
Ligustrum lucidum	St John's wort	Atractylodes lancea rhizome	Zuishi-torio	Dolichos seed	Nandin fruit	Japanese climbing fern
Safflower	Medical evodia	Saffron	Smilax rhizome	Japanese rush stem	Nandin leaf	Zedoary
Siberiang ginseng	Moutan bark	Phellodendron bark	Saussurea root	Hawthon	Catalpa fruit	Cassia seed
Asparagus root	Amomum seed	Alisma Rhizome	Achyranthes root(1)	Gentiana	Kaki persimmon leaf	Rakkyo
Nuphar rhizome	Hachimijio-gan	Trichosanthes root	Tartary buckwheat	Peucedani radix	Lycium leaf	Crested latesummer mint
Polygonatum thizome	Artemisia capillaris flower(2)	Rhubarb	Black soybean leaf	Ligusticum	Coffee senna	Sicklefruit fenugreek
Maca	Fragrant wormwood	Keishi-bukuryo-gan-ryo	Frankincense	Sappn wood	Dandelion	Processed ginger
Epimedium herb	Doku-dami	Toki-shakuyaku-san-ryo	Jujube seed	Gastrodia tuber	Japanese red elder	Ashitaba
Japanese angelica root	Japanese pepper	Boi-ogi-to	Forsythia Fruit	Rehmannia root steeped in sake	Field balm	Achyranthes root(2)
Chinese yam	Cornus fruit	Gorei-san-ryo	Angelica dahurica root	Nelumbo seed(2)	Common sage	Pharbitis seed
Cnidium rhizome	Nelumbo seed(1)	Hannge-shashin-to	Burdock fruit	Schisandra fruit	Tarragon	Cherokee rose
Chinese knotweed	Sinomenium stem(2)	Kakkon-to-ka-senkyu-shin'I	Asiassarum root	Coltsfoot flower	Red pepper	Beefsteak geranium
Silvervine	Leaf mustard	Daio-kanzo-to	Mangnolia flower	Roundleaf chastetree fruit	Anise seed	Polygala root
Cistanchis Harba	Lonicera leaf and stem	Shakuyaku-kanzo-to	Japanese peppermint	Saiko-ka-ryukotsu-borei-to	Allspice	Ginkgo leaf
Eucommia bark	Plantago seed	Bofu-tsusho-san	Corydalis tuber	Sokei-katsu-ketsu-to	Clove	German chamomile
Mugwort	Enmeisou	Seijo-bofu-to	Eagle-wood	Zokumei-to	Cayenne pepper	Field balm
Ganodermataceae	Anemarrhena rhizome	Hochu-ekki-to	Sinomenium stem(2)	Resty	Bay laurel	Chinaberry
Leonuri herba	Bupleurum root	Chorei-to	Polygonum root	Rumin A	Solomon's seal	Field horsetail
Turmeric	Greenbrier	Kakkon-to	Sophora root	Longan	Korean cherry	Hikiokoshi
Peony root	Coptis rhizome	Otsuji-to	Wax myrtle	Malt	Dwarf lilytur	Stellaria dichotoma
Rehmannia root	Scutellaria root	Hachimi-jio-gan-ryo	Lesser galangal rhizome	Hemp fruit	Cow herb	Fern rhizome
Swertia herb(1)	Pueraria root	Sho-saiko-to	Jasmine tea	Winter melon seed	Ume	Valerian
Saposhnikovia root	Geranium herb	Keishi-ka-ryukotsu-borei-to	Pu-ear-tea	Fritillaria bulb	Hedysarum polybotrys	Chuling
Cassia	Gardenia fruit	Mangnolia bark	Indian hemp	China berry	Mimosa	Conandron ramondioides
Clove	Ophiopogon tuber	Chuling	Fragrant tea	Cobra lily	Immature orange	Prunella spike
Fig	Areca	Poria sclerotium	Citrus unshiu peel	Digenea	Japanese torreya	False daisy
Sweet hydrangea leaf	Atractylon	Cherry bark	Oolong tea	Chinese sage(2)	Fortune drynaria	
Aloe	Fennel	Wood betony	Chrysanthemum flower	Sawtooth oak	Soybean	
Pine needle	Japanese gentian	Pinellia tuber	Japanese honeysuckle bud	Japanese pagoda tree	Scented rosewood	
Shiitake mushroom	Chinese sage(1)	Cimicifuga rhizome	Suwarowato	Massa medicata fermentat	Common rush	
Ginger	Jujube fruit	Pomegranate leaf	Tiger lily	Mallotus bark	Millettia	
Garlic	Ephedra herb	Guava leaf	Lithospermum root	Peach kernel	Galla rhois	
Green tee	Gambir	Chu-sho-to	Figuwato	Benzoin	Hairvein agrimony	
Chinese clematis root	Glehnia root	Epigallocatechin	Lindera roo	Japanese quassia wood	Variegated coral tree	
Uncaria hook	Glycyrrhiza	Epigallocatechin gallate	Schizonepeta spike	Bamboo shavings	Trichosanthes kernel	
Apricot	Unpolished rice	Catechin	Notopterygium rhizome	Polygonatum thizome	Clove Powder	
Loquart leaf	Rehmannia root	Epicatechin	Cnidium monnieri fruit	Swertia herb(2)	Japanese varelian	

time that extracts of Coptis rhizome and Phellodendron bark, and berberine inhibit the development of zygotes *in vitro* and full-term fetal development in the mouse.

Materials and Methods

All experiments and protocols were performed in strict accordance with the Guiding Principles for the Care and Use of Research Animals adopted by the Kinki University Committee on Animal Research and Bioethics. All chemicals were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO), unless otherwise stated.

Extracts of crude drugs

Ten grams each of Coptis rhizome and Phellodendron bark (Uchida Wakennyaku Co.,Tokyo, Japan) were ground to powder using a blender, and each powder sample was then boiled in 50 ml distilled water for 20 min. The extracts were centrifuged at 900 g for 5 min at 4ºC. The supernatant was decanted, freeze-dried, and stored at 4ºC until use.

Embryo culture

Superovulation was induced in adult hybrid F1 female mice (C57BL/6x DBA) by the injection of 5 IU equine chorionic gonadotrophin (eCG) and 5 IU human chorionic gonadotropin (hCG) 48 h apart. The mice were then mated with F1 males. Mated females were sacrificed 20 h after hCG injection, and eggs with cumulus cells were collected and treated with 300 NFU/ml hyaluronidase in M2 [8]. Denuded eggs with two pronuclei were used for the experiments. Five to ten zygotes were cultured in 10 μl KSOM/aa [9, 10] containing 0.01 μg/ml to 1,000 μg /ml Coptis rhizome or Phellodendron bark extract, or 0.0001 μg/ml to 10 μg/ml berberine chloride or berberine sulfate trihydrate (Wako Pure Chemical Industries, Ltd., Osaka, Japan) under 5% CO_2 in air at 37ºC for 5 days. The addition of Coptis rhizome or Phellodendron bark extracts, berberine chloride or berberine sulfate to the medium at 10 μg/ml had no effect on the pH (6.76 to 6.87) or osmolarity (250 to 251 mOsm).

To examine the effect of the timing of berberine treatment on the potential of zygotes to develop into blastocysts, we transferred 1-cell, 2-cell and 4-to 8-cell embryos and morulae cultured for 0, 22, 46 and 70 h, respectively, into the KSOM/aa supplemented with 0.1 μg/ml berberine chloride and cultured them for 118, 96, 72 and 48 h, respectively.

Effect of berberine administration on the in vivo development of embryos to blastocysts

Superovulation was induced in F1 females with eCG and hCG injections, and then the females were paired with F1 males and intramuscularly injected with 100 μg berberine chloride according to the injection schedules described below. The day on which a female was paired with a male was designated as day 0. Day 1 was the afternoon of the day when a vaginal plug was found, and day –1 means the day before day 0. The females received 100 μg berberine once a day on day 1 (Group 1; 1 injection), days 1 and 2 (Group 2; 2 injections), days 1 to 3 (Group 3; 3 injections), days –2 to 3 (Group 4; 6 injections), or days –10 to 3 (Group 5; 14 injections). All females were sacrificed on day 4 to recover embryos. Recovered eggs/embryos were classified as morulae, blastocysts, or others according to their developmental stage. Eggs/embryos classified as “others” included unfertilized eggs, and degenerated and retarded embryos. Control females received 0.1 ml distilled water following the same schedule as that used for the berberine administration.

Effect of berberine administration on fetal development

For experimental convenience, superovulation was induced in F1 females with injections of 2.5 IU eCG and 2.5 IU hCG 48 h apart, and the females were mated with F1 males and then received 100 μg berberine chloride for 6 days according to the schedule for Group 4 (6 injections, on days –2 to 3). Mated females were sacrificed to examine the numbers of live and dead fetuses on day 18.5 (day 0.5 was the morning of the day on which a vaginal plug was observed). Control females received 0.1 ml distilled water. Preliminary studies demonstrated that the proportion of blastocysts that developed *in vitro* into young after transfer to pseudopregnant recipients injected with 2.5 IU eCG and 2.5 IU hCG was not significantly different from that after transfer to naturally mated pseudopregnant recipients (36% vs. 46%).

Statistical analysis

The developmental data were analyzed by the chi-square test and data on the number of fetuses were analyzed by t-test. A *P*-value of less than 0.05 was considered to be statistically significant.

Table 2. Effect of the extracts of Coptis rhizome and Phellodendron bark on the *in vitro* development of mouse zygotes

Treatment	Concentrations (μg/ml)	No. of zygotes cultured	No. (%) of zygotes developed to	
			2-cell	blastocysts
Control	–	94	92 (98)[a]	76 (81)[a]
Coptis rhizome	0.01	61	60 (98)[a]	24 (39)[b]
	0.1	71	65 (92)[a]	2 (3)[c]
	1	73	66 (90)[a]	0
	10	71	14 (20)[b]	0
	100	30	0	0
	1,000	30	0	0
Phellodendron bark	0.01	43	43 (100)[a]	37 (86)[a]
	0.1	36	36 (100)[a]	21 (58)[b]
	1	20	20 (100)[a]	10 (50)[b]
	10	19	18 (41)[c]	3 (7)[c]
	100	26	0	0
	1,000	30	0	0

[a–c]: Values with different superscripts in the same columm differ significantly ($P < 0.05$).

Table 3. Effect of berberine on the *in vitro* development of mouse zygotes

Treatment	Concentrations (μg/ml)	No. of zygotes cultured	No. (%) of zygotes developed to			
			2-cell	4-8-cell	morulae	blastocysts
Control	–	94	93 (99)[a]	88 (94)[ab]	83 (88)[a]	72 (77)[a]
Berberine cloride	0.0001	43	42 (98)[a]	42 (98)[a]	37 (86)[ab]	35 (81)[a]
	0.001	53	51 (96)[a]	51 (96)[a]	47 (89)[a]	42 (79)[a]
	0.01	40	39 (98)[a]	34 (85)[b]	29 (73)[b]	1 (3)[b]
	0.1	43	43 (100)[a]	0	0	0
	1	41	5 (12)[b]	0	0	0
	10	41	0	0	0	0
Control	–	60	57 (95)[a]	57 (95)[a]	57 (95)[a]	39 (65)[a]
Berberine sulfate	0.001	63	58 (92)[a]	58 (92)[a]	58 (92)[a]	21 (33)[b]
	0.01	62	59 (95)[a]	50 (81)[b]	22 (35)[b]	0
	0.1	59	55 (93)[a]	17 (29)[c]	0	0
	1	55	25 (45)[b]	0	0	0
	10	55	0 (0)	0	0	0

[a–c]: Values with different superscripts in the same columm differ significantly ($P < 0.05$).

Results

Effect of Coptis rhizome, Phellodendron bark and berberine on the in vitro development of zygotes

Coptis rhizome at a concentration of more than 0.1 μg/ml and Phellodendron bark extracts at a concentration of more than 10 μg/ml almost completely inhibited the potential of zygotes to develop into blastocysts (Table 2). The development of zygotes to blastocysts was significantly reduced by Coptis rhizome at a concentration of 0.01 μg/ml and by Phellodendron bark at concentrations over 0.1 μg/ml.

The potential of zygotes to develop into blastocysts was almost completely inhibited when embryos were treated with 0.01 μg/ml berberine chloride or berberine sulfate (Table 3). The inhibitory effect of berberine was dose-dependent.

The inhibitory effects of berberine chloride on *in vitro* development were observed in embryos treated at all stages from the 1-cell to the morula. Even morulae treated with 0.1 μg/ml berberine chroride did not develop into blastocysts (Table 4).

Effect of berberine chloride administration on early embryonic development in vivo

The administration of berberine did not affect the

Table 4. Effect of the timing of berberine chloride treatment on the development of mouse embryos

Group	Timing of treatment	No. of embryos cultured	No. (%) of embryos developed to			
	Developmental stage (h after culture)		2-cell	4-8-cell	morulae	blastocysts
Control	1-cell (–)	70	70 (100)	70 (100)[a]	66 (94)[a]	66 (94)
Berberine	1-cell (0)	48	44 (92)	3 (6)[b]	0	0
	2-cell (22)	29	–	1 (3)[b]	0	0
	4-8-cell (46)	47	–	–	8 (17)[b]	0
	morula (70)	47	–	–	–	0

Berberine chloride was added to the culture medium at the concentration of 0.1 μg/ml. [a–b]: Values with different superscripts in the same columm differ significantly ($P < 0.05$).

Table 5. Effect of berberine chloride administration on the *in vivo* development of embryos

Group	Administration period (day)	No. of females paired with males	No. (%) of mated females (with vaginal plug)	No. of eggs/ embryos recovered (average per mated female)	No. (%) of revovered embryos at		
					morulae	blastocysts	others
G3: DW	3	14	10 (71)	334 (33.4)	124 (37)[a]	145 (43)[a]	65 (19)[a]
G1: Berberine	1	9	6 (67)	173 (28.8)	49 (28)[b]	95 (55)[b]	29 (17)[a]
G2: Berberine	2	8	6 (75)	126 (21.0)	37 (29)[a]	41 (33)[c]	48 (38)[b]
G3: Berberine	3	14	13 (93)	366 (28.2)	105 (29)[b]	138 (38)[c]	123 (34)[b]
G6: DW	6	14*	11 (85)***	131 (11.9)	54 (41)	46 (35)[a]	31 (24)[a]
G6: Berberine	6	25**	18 (82)***	123 (6.8)	50 (41)	12 (10)[b]	61 (50)[b]
G14: DW	14	7*	5 (83)	103 (20.6)	23 (22)	68 (66)[a]	12 (12)
G14: Berberine	14	11*	9 (90)	164 (18.2)	62 (38)	68 (41)[b]	34 (21)

[a–c]: Values with different superscripts in the same columm and the same experiment differ significantly ($P < 0.05$). *One female in each group died before mating. **Three females died before mating. ***One female in each group died before embryo recovery.

Table 6. Effect of berberine chloride administration on fetal development

Treatment	No. of females paired with males	No. of females mated (with vaginal plug)	No. (%) of mated females with fetuses	No. of fetuses	
				live (average ± SD)	dead
DW	16	15 (94)	9 (60)	156 (17.3 ± 6.7)[a]	4
Berberine	16	16 (100)	10 (63)	121 (12.1 ± 3.7)[b]	5

[a–b]: Values with different superscripts differ significantly ($P < 0.05$).

proportions of mated females (Table 5). When 100 μg berberine was intramuscularly injected for 2, 3, 6 or 14 days, the percentage of recovered embryos at the blastocyst stage in each group was significantly smaller than that in controls (10% to 41% vs. 35% to 66%, respectively). A few mice died following berberine injection, before or after mating (4 out of 25 and 1 out of 11 mice), or following distilled water injection (2 out of 14 and 1 out of 7 mice) in Group 6 and Group 14, respectively.

Table 6 shows the effect of berberine injection on the development to full-term. The proportions of females mated (100% vs. 94% in the control group) and with fetuses (63% vs. 60% in the control group) on day 18.5 did not differ significantly from the control group. The average number of live fetuses in the berberine-administered females was significantly lower than that of the control females (12.1 vs. 17.3, respectively).

Discussion

Coptis rhizome and Phellodendron bark are widely used for the treatment of gastroenteritis, diarrhea, cholera, and human immunodeficiency virus (HIV) in

traditional Chinese medicine [6, 7], and herbal medicines containing Coptis rhizome or Phellodendron bark are commercially available in Japan. Berberine is an alkaloid component of Coptis rhizome and Phellodendron bark [7] that is used to treat diseases such as hypotension, vasorelaxation, diarrhea, respiratory infection, HIV and human cancer cells [7, 11]. Although berberine administration to pregnant women is not recommended, because berberine displaces bilirubin from serum-binding proteins, causing jaundice, kernicterus, and brain damage in infants, there are no reports on the toxicity of berberine at clinically relevant doses [7]. When berberine chloride dihydrate was administered in the feed to pregnant mice on days 6 to 17 of gestation, 33% of mice administered a high dosage of berberine (1,000 mg/kgBW/day) died from unknown causes, but prenatal mortality, average litter size, and percentage of male fetuses were not affected [12].

To date, although there have been a number of pharmacologic and therapeutic studies of Coptis rhizome, Phellodendron bark and berberine [7, 13], their effects on fertility have not been reported. We found that water-soluble extracts of Coptis rhizome, and Phellodendron bark inhibited the development of mouse zygotes to blastocysts *in vitro*. We also found that berberine, a component of both natural products, inhibited the development of mouse zygotes. Moreover, intramuscular injection of berberine decreased the frequency of blastocysts and full-term fetuses. To our knowledge, this is the first report that Coptis rhizome, Phellodendron bark, and berberine inhibit the development of mouse zygotes.

The precise mechanisms by which both natural products and berberine inhibit the development of zygotes are not clear. Because the development of 1-cell, 2-cell, 4 to 8-cell, and morula-stage embryos to later stages was inhibited after the administration of berberine chloride, its inhibitory effect is likely related to cell division of blastomeres rather than embryonic genome activation, which occurs at the 2-cell stage in the mouse [14]. Berberine inhibits the growth of various types of cancer cells by inhibiting DNA topoisomerase I and by inducing cell-cycle arrest and apoptosis, mainly through the caspase-3 or Fas/FasL signaling pathway [13, 15, 16]. Serafim *et al.* [17] reported that berberine at low doses promotes G1 arrest but at higher doses results in G2 arrest. In the present study, we did not examine the cell cycle stage or apoptosis in development-arrested embryos after berberine treatment. Because most zygotes treated with 0.01 μg/ml berberine chloride developed to the morula stage, but only a few berberine-treated embryos developed into blastocysts, and morulae treated with 0.1 μg/ml berberine chloride did not develop to blastocysts, it is possible that berberine inhibits the cell differentiation in the morula.

The present study demonstrated that berberine administered to female mice once a day for 2 to 14 days after superovulation significantly decreased the proportion of recovered blastocysts and the fetal rate compared with those of control females. Although the precise mechanism is not clear, berberine administered intramuscularly may travel to oviducts and uteri through epithelial cells and inhibit embryonic development. Unlike gossypol [4], the toxicity of berberine except at an unusually high dosage has not been reported. The findings of the present study indicate the potential use of berberine as a contraceptive for animals. However, effective procedures to enhance the transport of berberine to the genital tracts have to be developed, because the inhibition of fetal development is not complete. Follow-up *in vivo* studies are needed to demonstrate that fertility can be restored. Further studies should be conducted to reveal the mechanism of inhibitory action of berberine on the embryonic development.

Acknowledgements

This work was supported by the Ministry of Education, and Culture, Sports, Science and Technology, Japan (16200030, 20650063, 21028022).

References

1) Tsunoda, Y. and Kato, Y. (2006): Cloning in cattle. In: Epigenetic Risks of Cloning (Inui, A., ed.), pp.33–57, Taylor & Francis, Boca Raton.
2) Chang, M.C. (1978): Development of the oral contraceptives. Am. J. Obstet. Gynecol., 132, 217–219.
3) Galvao, K.N., Sanntos, J.E.P., Coscioni, A.C., Juchem, S.O., Chebel, W.M., Sischo,W.M. and Villasenor, M. (2006): Embryo survival from gossypol-fed heifers after transfer to lactating cows treated with human chorionic gonadotropin. J. Dairy Sci., 89, 2056–2064.
4) Randel, R.D., Chase, C.C. Jr. and Wyse, S.J. (1992): Effects of gossypol and cottonseed products on reproduction of mammals. J. Anim. Sci., 70, 1628–1638.
5) Brocas, C., Rivera, R.M., Paula-Lopes, F.F., McDowell, L.R., Calhoun, M.C., Staples, C.R., Wilkinson, N.S., Boning, A.J., Chenoweth, P.J. and Hansen, P.J. (1997): Deleterious actions of gossypol on bovine spermatozoa, oocytes, and embryos. Biol. Reprod., 57, 901–907.

6) Chan, C-O., Chu, C-C., Mok, D.K-W. and Chau, F-T. (2007): Analysis of berberine and total alkaloid content in *Cortex Phellodendri* by near infrared spectroscopy (NIRS) compared with high-performance liquid chromatography coupled with ultra-viable spectrometric detection. Analyt. Chim. Acta., 592, 121–131.
7) Imanshahidi, M. and Hosseinzadeh, H. (2008): Pharmacological and therapeutic effects of *Berberis vulgaris* and its active constituent, berberine. Phyt. Res., 22, 999–1012.
8) Fulton, B.P. and Whittingham, D.G. (1978): Activation of mammalian oocytes by intacellular injection of calcium. Nature, 273, 149–151.
9) Erbach, G.T., Lawittsm, J.A., Papaioannou, V.E. and Biggers, J.D. (1994): Differential growth of the mouse preimplantation embryo in chemically defined media. Biol. Reprod., 50, 1027–1033.
10) Kato, Y. and Tsunoda, Y. (1994): Effect of culture density of mouse zygotes on the development in vitro and in vivo. Theriogenology, 41, 1315–1322.
11) Yu, F-S., Yang, J-S., Lin, H-J., Yu, C-S., Tan, T-W., Lin, Y-T., Lin, C-C., Lu, H-F. and Chung, J-G. (2007): Berberine inhibits WEHI-3 leukemia cells in vivo. In Vivo, 21, 407–412.
12) Jahnke, G.D., Price, C.J., Marr, M.C., Myers, C.B. and George, J.D. (2006): Developmental toxicity evaluation of berberine in rats and mice. Birth Defects Res. (Part B), 77, 195–206.
13) Tang, J., Feng, Y., Tsao, S., Wang, N., Curtain, R. and Wang, Y. (2009): Berberine and Coptidis Rhizoma as novel antineoplastic agents: A review of traditional use and biomedical investigations. J. Ethnopharm., 126, 5–17.
14) Kidder, G.M. (1992): The genetic program for preimplantation development. Develop. Genetics, 13, 319–325.
15) Li, X.K., Motwani, M.T., William, B., William, S. and Gary, K.S. (2000): Huanglian, a Chinese herbal extract, inhibits cell growth by suppressing the expression of cyclin B1 and inhibiting CD2 kinase activity in human cancer cells. Mol. Pharm., 58, 1287–1293.
16) Kuo, C.L., Chi, C.W. and Liu, T.Y. (2005): Modulation of apoptosis by berberine through inhibition of cyclooxygenase-2 and Mcl-1 expression in oral cancer cells. In Vivo, 19, 247–252.
17) Serafim, T.L., Olivera, P.J., Sardao, V.A., Perkins, E., Parke, D. and Holy, J. (2008): Different concentrations of berberine result in distinct cellular localization patterns and cell cycle effects in a melanoma cell line. Cancer Chem. Pharm., 61, 1007–1018.