NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 49, 2016 (3): 132–136

ISSN 1337-9984

Short communication

THE EFFECT OF CURCUMA LONGA PLANT EXTRACT ON THE RABBIT EMBRYO DEVELOPMENT *IN VITRO*

M. FÖLDEŠIOVÁ[1]*, A. BALÁŽI[1], P. CHRENEK[1,2]

[1]NPPC – Research Institute for Animal Production Nitra, Slovak Republic
[2]Slovak University of Agriculture, Faculty of Biotechnology and Food Science, Nitra, Slovak Republic

ABSTRACT

The aim of this study was to evaluate the effect of *Curcuma longa* (*CL*) root extract on the rabbit embryo development *in vitro*. Totally 113 pronuclear stage zygotes were used in this experiment. Zygotes were divided into 4 groups: control (C; n = 28) and three experimental groups (E1, E2, E3; n = 31, 26 and 39, resp.) with addition of different concentrations of *Curcuma longa* extract to the culture medium (E1- 0.1 µg-ml^{-1}; E2- 0.01 µg.ml^{-1}; E3- 0.001 µg.ml^{-1}). Zygotes were cultured up to the blastocyst stage (120 h) in 5 % CO_2 at 37.5 °C. At the end of culture period the blastocysts were stained with DAPI fluorochrome for the total cell number determination. Evaluation of embryo developmental potential showed, that higher blastocyst rate was observed in the E2 (61.5 %) and E3 (60 %) groups compared to the control group (46.4 %). In the group with highest *CL* concentration in culture (E1- 0.1 µg.ml^{-1}) embryo development was stopped at the morula stage. In this group also the lowest ($P < 0.001$) number of cleaved embryos (19.4 %) compared to the control (60.7 %) and E3 group (82.1 %) was recorded. There were no differences in the blastocysts total cell number among the groups with lower *CL* concentrations (E2 77.81 ± 13.6; E3 89.25 ± 15.94) and control group (82.23 ± 21.75). On the basis of our results we suppose that *Curcuma longa* affects rabbit embryo development in a dose-dependent manner. Although lower concentrations showed positive effect, the highest concentration blocked embryo development at morula stage. It is necessary to determine since which concentration *Curcuma longa* may be toxic for normal embryonic development.

Key words: rabbit; embryos; *Curcuma longa* extract; *in vitro* development; DAPI staining

INTRODUCTION

Medical plants are widely used as a source of remedies for the treatment and prevention of many diseases as alternative therapeutic and medical tools (Kaur and Mondal, 2014). Natural products from some plants are used in pharmaceutical preparations either as pure compounds or as extracts (Araújo and Leon, 2001). One of them is *Curcuma longa* Linn. (*Zingiberaceae* family), well-known as turmeric, broadly grown in tropical areas of Asia and Central America (Ammon, 1991). This rhizome in powder form is widely used as a food additive for impart flavour and a yellow colour (Miquel *et al.*, 2002). The major constituent, curcumin (diferuloylmethane) is the most important fraction of *Curcuma longa* (Araújo and Leon, 2001). It has been already demonstrated, that curcuminoids have anti-atherosclerotic (Olszanecki *et al.*, 2005), anti-diabetic (Nabavi *et al*., 2015), anti-mutagenic, anti-cancer (Goel *et al.*, 2008), antioxidant (Huang *et al*., 1994; Nishiyama *et al.*, 2005; Wei *et al*., 2006; Kumar *et al*. 2007), anti-bacterial (Park *et al*., 2005), anti-inflammatory

***Correspondence:** E-mail: mt.foldesiova@gmail.com
Martina Földešiová, NPPC – Research Institute for Animal Production Nitra, Institute of Farm Animal Genetics and Reproduction , Hlohovecká 2, 951 41 Lužianky, Slovak Republic
Tel.: +421 37 6546 335

Received: June 20, 2016
Accepted: August 17, 2016

and anti-fertility (Mishra and Singh, 2009; Ammon and Wahl, 1991; Lantz *et al.*, 2005) activities. Garg *et al.* (1974; 1978) found out, that aqueous extracts of turmeric rhizome show complete inhibition of embryo implantation in rats when fed orally. Curcumin has the potential for the use in development of novel intravaginal contraceptive (Rithaporn *et al.*, 2003). Thakur *et al.* (2009) observed significant anti-fertilizing activity and decreasing of FSH and LH levels in blood plasma of albino rat females after oral administration of aqueous or ethanolic extracts from *Curcuma longa*. Many studies showed strong correlation between antioxidant activity and fertility (Ruder *et al*., 2009), as well as between free radical accumulation and reduction in fertility (Behrman *et al.*, 2001). The aim of this study was to examine the effect of *Curcuma longa* Linn. on rabbit embryo development *in vitro*.

MATERIAL AND METHODS

Animals and superovulation

The treatment of the animals was approved by the Ministry of Agriculture and Rural Development of the Slovak Republic, no. SK P 28004 and Ro 1488/06-221/3a. Sexually mature New Zealand White rabbit does from the Department of Small Farm Animals, APRC Nitra were used in this experiment. Superovulation of rabbit does was induced by intramuscular application of 50 IU PMSG (SERGON, Bioveta, a. s. Ivanovice na Hané, Czech Republic) and after 48 hours by 100 µl of HCG (Supergestran, Nordic Pharma s.r.o. Jesenice, Czech Republic) per doe. Before the HCG injection, all rabbit does were artificially inseminated by heterospermic dose of rabbit semen (0.5 ml/doe).

Egg recovery, culture and staining

At 19-20 h *post-coitum*, rabbit does were humanely slaughtered by the electrical stunning (Relco, Gewiss, Milano, Italy, alternating current 0.3 A/female, frequency 50 Hz, exposition 4s) and reproductive organs were expertly dissected. The pronuclear stage eggs were flushed from the oviducts with PBS (Gibco, Auckland, New Zealand) and subsequently morphologically evaluated. The selected eggs were placed into 4-well dishes (Nunc, Roskilde, Denmark) containing 500 µl of k-DMEM medium (Gibco) supplemented with three different concentrations of *Curcuma longa* (*CL*) extract (E1- 0.1 µg.ml^{-1} *CL*; E2- 0.01 µg.ml^{-1}; E3- 0.001 µg.ml^{-1}) and cultured up to 120 hours *post-coitum* in 5 % CO_2 at 37.5 °C (the time point to reach the blastocyst stage). After the culture, embryos were washed in PBS with polyvinylpyrrolidone (PBS-PVP, 4 mg.ml^{-1}) for 3 x 5 min, stained with 4 µl of Vectashield mounting medium with DAPI (Vector Laboratories, Burlingames, CA, USA) and mounted between the microslide and coverslip. Total cell number was counted under a Zeiss fluorescence microscope equipped with a specific wave-length filter (Fig. 1).

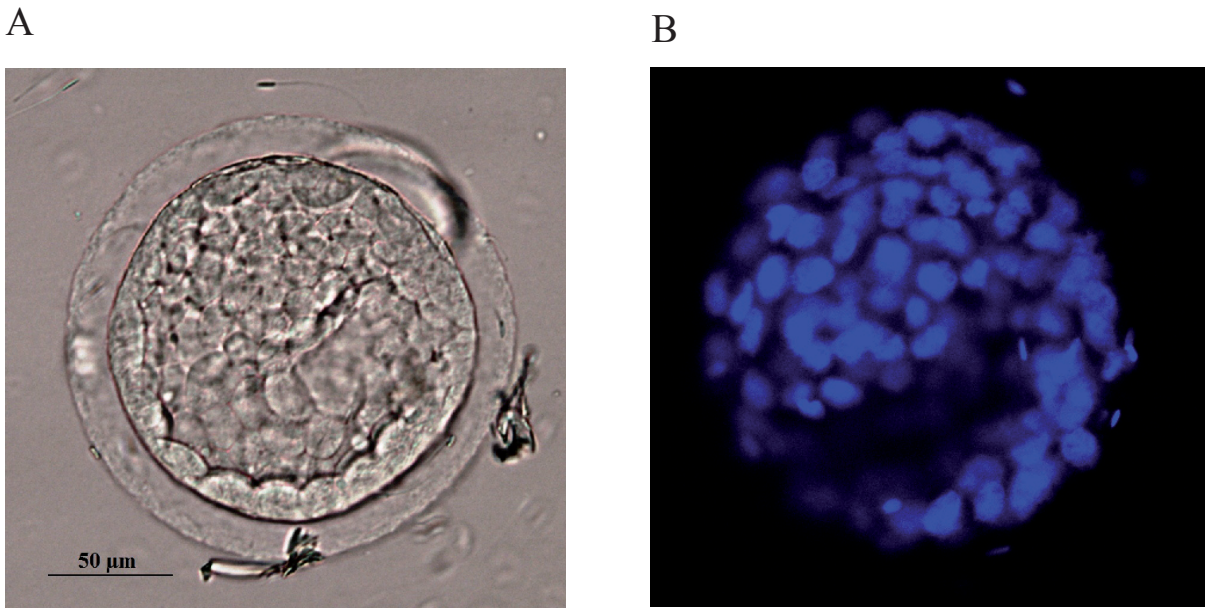

Fig. 1: Representative image of the embryo cultured with the lowest concentration (0.001 µg.ml^{-1}) of *Curcuma longa* extract. A: light microscopy; B: fluorescence microscopy (cells stained by DAPI fluorochrome)

Statistical analysis

The data were analysed by Pearson's Chi-square test.

RESULTS AND DISCUSSION

To examine the effect on embryo development we cultured rabbit pronuclear stage eggs in the medium enriched with three different concentrations of *Curcuma longa*.

The highest concentration of *CL* (E1 group) had negative effect on the embryo development, as was shown by decreasing ($P < 0.001$) cleavage rate (19.4 %) compared to the control and the other experimental groups (C: 60.7 %, E2: 69.2, E3: 82.1 %).

The lowest concentration of *CL* (E3 group) increased ($P < 0.001$) cleavage rate (82.1 %) when compared to control. Development to the blastocyst stage was completely stopped in the E1 group, whereas blastocyst rates in the E2 (61.5 %), E3 (60 %) and C (46.4 %) groups were not statistically different (Chi-square test).

Negative effect of *Curcuma longa* on reproduction was already reported on granulosa cells of porcine ovary, where it inhibited proliferation (accumulation of PCNA) and induced apoptosis (accumulation of bax) (Kádasi *et al.*, 2012; Voznesenska *et al*., 2010; Bhaumik *et al.*, 1999). In our study, the highest concentration (0.1 μg.ml^{-1}) of *CL* in the culture stopped embryo development at the morula stage. Possible explanation could be the stimulation of apoptotic process due to the toxicity of mentioned concentration in culture. At the blastocyst stage apoptosis is responsible for the elimination of undesirable cells during the normal embryonic development (Hardy *et al.*, 2003). However, increased occurrence of apoptosis before or during the blastocyst stage probably removes important cell lineages, what might negatively affect embryonic development and lead to embryo degeneration (Long *et al.*, 2000). Although, the objective of our study was not an evaluation of apoptosis incidence, the similar total cell number in each group indicates that there is no a developmental delay and increased apoptosis incidence. A similar conclusion was reported by Chen *et al.* (2010), who applied *Curcuma longa* to mouse embryo culture. On the basis of the blastocyst development evaluation by differential staining, the authors found that higher concentration (24 μM.ml^{-1}) of the *CL* extract induced apoptosis in the ICM but not in trophoblastic cells. Nevertheless, lower concentrations (6 and 12 μM.ml^{-1}) did not affect the apoptosis incidence or cell number. Likewise, in our study similar blastocyst cell numbers in the groups with lower *CL* concentration (E2: 77.81 ± 13.6; E3: 89.25 ± 15.94) and control group (82.23 ± 21.75) were found. Because none of the embryos were developed to blastocyst stage in the group with the highest concentration, counting of the total cell number in this group was not performed.

CONCLUSION

According to our results we can conclude that the highest concentration of *Curcuma longa* root extract added to culture medium negatively affects embryo cell number and terminates embryo development at the morula stage.

ACKNOWLEDGEMENT

This study was supported by the grants APVV-0854-11, KEGA-011UPJŠ-412014 and VEGA 1/0611/15.

Table 1: Effect of different *Curcuma longa* concentrations on rabbit embryo development

Group	Number of embryos (n)	Cleavage rate (%)	Morula rate (%)	Blastocyst rate (%)
C	28	17/60.7^{b}	4/14.3^{c}	13/46.4^{f}
E1	31	6/19.4^{a}	6/19.4^{c}	0/0^{e}
E2	26	18/69.2^{b}	2/7.7^{d}	16/61.5^{f}
E3	39	32/82.1^{b}	7/17.9^{c}	23/60.0^{f}

Level of significance: $P<0.001$ a:b and e:f; $P<0.01$c:d

C- control group, E1- 0.1 μg-ml^{-1} of *Curcuma longa* in the culture, E2- 0.01 μg.ml^{-1} of *Curcuma longa* in the culture, E3- 0.001 μg.ml^{-1} of *Curcuma longa* in the culture

REFERENCES

AMMON, H. P. − WAHL, M. A. 1991. Pharmacology of *Curcuma longa* L. *Planta Medica*, vol. 57 (1), 1991, p. 1−7.

ARAÚJO, C. A. C. − LEON, L. L. 2001. Biological activites of *Curcuma longa* L. *The Memórias do Instituto Oswaldo Cruz*, vol. 96 (5), 2001, p. 723−728.

BEHRMAN, H. R. − KODAMAN, P. H. − PRESTON, S. L. − GAO, S. 2001. Oxidative stress and the ovary. *Journal of Society for Gynecologic Investigation*, vol. 8 (1), 2001, p. 40−42.

BHAUMIK, S. − ANJUM, R. − RANGARAJ, N. − PARDHASARADHI, B. V. − KHAR, A. 1999. Curcumin mediated apoptosis in AK-5 tumor cells involves the production of reactive oxygen intermediates. *FEBS Letters*, vol. 456 (2), 1999, p. 311−314.

GARG, S. K. 1974. Effect of *Curcuma longa* (rhizomes) on fertility in experimental animals. *Planta Medica*, vol. 26 (3), 1974, p. 225−227.

GARG, S. K. − MATHUR, V. S. − CHAUDHURY, R. R. 1978. Screening of Indian plants for antifertility activity. *Indian Journal of Experimental Biology*, vol. 16 (10), 1978, p. 1077−1079.

GOEL, A. − KUNNUMAKKARA, A. B. − AGGARWAL, B. B. 2008. Curcumin as "Curecumin": from Kitchen to clinic. *Biochemical Pharmacology*, vol. 75 (4), 2008, p. 787−809.

HARDY, K. − STARK, J. − WINSTON, R. M. 2003. Maintenance of the inner cell mass in human blastocysts from fragmented embryos. *Biology of Reproduction*, vol. 68 (4), 2003, p. 1165−1169.

HUANG, M. T. − LOU, Y. R. − MA, W. − NEWMARK, H. L. − REUHL, K. R. − CONNEY, A. H. 1994. Inhibitory effects of dietary curcumin on forestomach, duodenal and colon carcinogenesis in mice. *Cancer Research*, vol. 54 (22), 1994, p. 5841−5847.

CHEN, CH. CH. − HSIEH, M. S. − HSUUW, Y. D. − HUANG, F. J. − CHAN, W. H. 2010. Hazardous Effects of Curcumin on Mouse Embryonic Development through a Mitochondria-Dependent Apoptotic Signaling Pathway. *International Journal of Molecular Science*, vol. 11 (8), 2010, p. 2839−2855.

KAUR, S. − MONDAL, P. 2014. Study of Total Phenolic and Flavonoid Content, Antioxidant Activity and Antimicrobial Properties of Medicinal Plants. *Journal of Microbiology and Experimentation*, vol. 1 (1), 2014, p. 1−6.

KÁDASI, A. − SIROTKIN, A. V. − MARUNIAKOVA, N. − KOLESÁROVÁ, A. − BULLA, J. − GROSSMANN, R. 2012. The effect of curcumin on secretory activity, proliferation and apoptosis of the porcine ovarian granulosa cells. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 2 (1), 2012, p. 20349−20357.

KUMAR, P. − PADI, S. S. − NAIDU, P. S. − KUMAR, A. 2007. Possible neuroprotective mechanisms of curcumin in attenuating 3-nitropropionic acid-induced neurotoxicity. *Methods and Findings in Experimental a Clinical Pharmacology*, vol. 29 (1), 2007, p. 19−25.

LANTZ, R. C. − CHEN, G. J. − SOLYOM, A. M. 2005. The effect of turmeric extracts on inflammatory mediator production. *Phytomedicine*, vol. 12 (6-7), 2005, p. 445−452.

LONG, L. H. − CLEMENT, M. V. − HALLIWELL, B. 2000. Artifacts in cell culture: Rapid generation of hydrogen peroxide on addition of (-)-epigallocatechin, (-)-epigallocatechin gallate, (+)-catechin, and quercetin to commonly used cell culture media. *Biochemical and Biophysical Research Communications*, vol. 273 (1), 2000, p. 50−53.

MIQUEL, J. − BERND, A. − SEMPRE, J. M. − DÍAZ-ALPERI, J. − RAMÍREZ, A. 2002. The curcuma antioxidants: pharmacological effects and prospects for future clinical use. A review. *Archives of Gerontology and Geriatrics*, vol. 34 (1), 2002, p. 37−46.

MISHRA, R. K. − SINGH, S. K. 2009. Reversible antifertility effect of aqueous rhizome extract of *Curcuma longa* L. in male laboratory mice. *Contraception*, vol. 79 (6), 2009, p. 479−487.

NABAVI, S. F. − THIAGARAJAN, R. − RASTRELLI, L. − DAGLIA, M. − SOBARZO-SANCHEZ, E. − ALINEZHAD, H. − NABAVI, S. M. 2015. Curcumin: A Natural Product for Diabetes and its Complications. *Current topics in medicinal chemistry*, vol. 15 (23), 2015, p. 2445−2455.

NISHIYAMA, T. − MAE, T. − KISHIDA, H. − TSUKAGAWA, M. − MIMAKI, Y. − KURODA, M. 2005. Curcuminoids and sesquiterpenoids in turmeric (*Curcuma longa* L.) suppress an increase in blood glucose level in type 2 diabetic KK-Ay mice. *Journal of Agricultural and Food Chemistry*, vol. 53 (4), 2005, p. 959−963.

OLSZANECKI, R. − AWIEŃ, J. − GAJDA, M. − MATEUSZUK, L. − GEBSKA, A. − ORABIOWSKA, M. − CHŁOPICKI, S. − KORBUT, R. 2005. Effect of curcumin on atherosclerosis in apoE/LDLR-double knockout mice. J*ournal of Physiology and Pharmacology*, vol. 56 (4), 2005, p. 627−635.

PARK, B. S. − KIM, J. G. − KIM, M. R. 2005. *Curcuma longa* L. constituents inhibit sortase A and staphylococcus aureus cell adhesion to fibronectin. *Journal of Agricultural and Food Chemistry*, vol. 53 (23), 2005, p. 9005−9009.

RITHAPORN, T. − MONGA, M. − RAJASEKHARAN, M. 2003. Curcumin: a potential vaginal contraceptive. *Contraception*, vol. 68 (3), 2003, p. 219−223.

RUDER, E. H. − HARTMAN, T. J. − GOLDMAN, M. B. 2009. Impact of oxidative stress on female fertility. *Current Opinion in Obstetrics and Gynecology*, vol. 21 (3), 2009, p. 219−222.

THAKUR, S. − BAWARA, B. − DUBEY, A. − NANDINI, D. − CHAUHAN, N. S. − SARAF, D. K. 2009. Effect of *Carum carvi* and *Curcuma longa* on hormonal and reproductive parameter of female rats. *International Journal of Phytomedicine*, vol. 1 (1), 2009, p. 31−38.

VOZNESENSKA, T. I. − BRYZHINA, T. M. − SUKHINA, V. S. − MAHKOHON, N. V. − ALEKSIEIEVA, I. M. 2010. Effect of NF-kappaB activation inhibitor curcumin on the oogenesis and follicular cell death in immune ovarian failure in mice. *Fiziolohichnyĭ zhurnal*, vol. 56 (4), 2010, p. 96−101.

WEI, Q. Y. − CHEN, W. F. − ZHOU, B. − YANG, L. − LIU, Z. L. 2006. Inhibition of lipid peroxidation and protein oxidation in rat liver mitochondria by curcumin and its analogues. *Biochimica Biophysica Acta*, vol. 1760 (1), 2006, p. 70−77.