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# EFFECT OF AMYGDALIN ON ANEUPLOIDY INCIDENCE IN RABBIT

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## ABSTRACT

The aim of this study was to examine possible effect of amygdalin substance applied to rabbit by different ways on the occurrence of aneuploidy. Totally 30 adult females of the New Zealand White line were used in the experiment. Animals were divided into four experimental groups and one control group, each consisted of five individuals. In groups P1 and P2, isolated amygdalin substance was administered intramuscularly (P1: 0.6 mg.kg$^{-1}$; P2: 3 mg.kg$^{-1}$ of body weight), whilst in P3 and P4 the animals were fed by a mixture of commercial diet and apricot seeds (P3: 60 mg.kg$^{-1}$; P4: 300 mg.kg$^{-1}$ of body weight). Experimental treatment lasted one month of continuous amygdalin application (one experimental dose applied per day in each group). Aneuploidy assay was performed on peripheral blood lymphocytes arrested at the metaphase stage by colcemid solution (0.6μg.ml$^{-1}$). The average occurrence of diploidy versus aneuploidy (presented by hypodiploid nuclei) was detected as follows: P1- 76 % vs 21.6 %; P2 - 72.8 % vs 27.2 %; P3 - 82.4 % vs 17.6 %; P4 - 70.4 % vs 29.6 %; Control - 70.4 % vs 26.8 %. Low incidence of polyploid cells was found in P1 (2.4 %) and control (2.8 %) groups. In conclusion, no significant effect of amygdalin on aneuploidy occurrence in rabbit blood cells was found.

**Key words:** amygdalin; aneuploidy; lymphocyte; chromosome; rabbit

## INTRODUCTION

Amygdalin, the substance hidden under various nicknames, including: vitamin B17, nitriloside, mandelonitrile, etc. (Fukuda *et al*., 2003) is widely distributed in plants, especially in the *rosaceous* plant seed, such as: apricot and peach (Santos Pimenta *et al*., 2014). It can hydrolyze and generate prunasin and mandelonitrile under the glucosidase action, such as amygdalase and prunase, and ultimately decomposed into benzaldehyde and hydrocyanic acid. Amygdalin itself is non-toxic, but its production of hydrocyanic acid decomposed by some enzymes is poisonous substance (Suchard *et al*., 1998). There are number of studies describing the effect of amygdalin and derived substances on the recipient body. For example: anti-tussive and anti-asthmatic effects by the amygdalin decomposition to hydrocyanic acid which could inhibit the respiratory center to a certain level (Chang *et al*., 2005; Do *et al*., 2006); effects on the digestive system by inhibition of the pepsin activity (Song and Xu, 2014); analgesic effect by inhibiting prostaglandins E2 and nitric oxide synthesis (Yang *et al.*, 2007; Paoletti *et al.*, 2013); promoting apoptosis of human renal fibroblast by enhancing the activity of type I collagenase (Guo *et al.*, 2013); improving the immune function of organism by the significant increase of polyhydroxyalkanoates inducing human peripheral blood T lymphocyte proliferation (Baroni *et al*., 2005); the anti-tumor effect of amygdalin presented by the hydrocyanic acid, which is an anti-tumor compound formed from the amygdalin decomposition (Kwon *et al.*, 2003). In other studies, amygdalin significantly inhibited sperm hyaluronidase activity and spermatozoa motility of bull sperm *in vitro* (Tanyildizy and Bozkurt, 2004). Recent data indicated that amygdalin reduced proliferation potential, decreased mitochondrial activity of cervical cancer cells, accumulated cells in the G1-phase and led to their death

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(Jarocha and Majka, 2011).

Various ways of amygdalin application play a different role in recipient organism, what was confirmed by Moertel *et al*. (1981), who demonstrated in human, that intravenous infusion of amygdalin produced neither cyanidemia nor signs of toxicity, but oral administration resulted in significant blood cyanide levels.

Cytogenetic analysis plays an important role in livestock breeding. The chromosomal abnormalities, caused by various factors, are generally divided into structural and/or numerical changes. Commonly observed aneuploidies are hypo (loss) and hyper (gain) haploidy, as a result of meiotic and mitotic non-disjunction events (Goepfert *et al*., 2000). Normal organisms are characterised by the presence of aneuploid cells at very low frequency. In rabbits, the rate of aneuploidy varies depending on the tissue of origin, from 5 % in *in vitro* fertilized oocytes (Asakawa *et al*., 1988), 16-18 % in blood cells (Zartman and Fechheimer, 1967; Parkanyi, 1981), 35-51 % in bone marrow (Parkanyi, 1981; Curlej *et al*., 2007), up to 56-83 % in embryos (Austin, 1967; Shi *et al*., 2004; Curlej *et al*., 2010). Diseases, assisted reproductive technologies, or genetic manipulations might cause an increase in the incidence of aneuploidy (Hegedus *et al*., 2008).

The present study was focused to reveal the effect of various amygdalin concentrations, administrated by different ways, to evoke the aneuploidy on the rabbit model. To achieve the paper goals chromosomes were recovered from lymphocyte's nuclei isolated from the peripheral blood.

## MATERIAL AND METHODS

### Animals

A total of 30 adult rabbit females (20 individuals assigned to the experimental groups P1-P4 and 10 rabbits selected to control group) of the New Zealand White line were used in the experiment. The animals were bred at the farm of the Research Institute for Animal Production, NAFC Nitra. Prior to start of the experiment, animals were selected according to similar age and good health status which was continuously monitored from the start up to the end of the experimental treatment. Environmental conditions were maintained using a control system for the light (16:8 light/dark photoperiod), air ventilation and the temperature (18-25 °C). Animals were fed *ad libitum* with treated/non-treated commercial rabbit diet. The experiment was lasted one month of continuous amygdalin application (one experimental dose applied per day in each group) as described in the text below.

### Experimental groups

Group P1 (n = 5): intramuscular application of isolated amygdalin substance at the final concentration of 0.6 $mg.kg^{-1}$ of body weight.

Group P2 (n = 5): intramuscular application of isolated amygdalin substance at the final concentration of 3 $mg.kg^{-1}$ of body weight.

Group P3 (n = 5): feeding by a mixture of commercial diet and apricot seeds at the final concentration of 60 $mg.kg^{-1}$ of body weight.

Group P4 (n = 5): feeding by a mixture of commercial diet and apricot seeds at the final concentration of 300 $mg.kg^{-1}$ of body weight.

### Chromosome preparation

Venous blood samples were collected aseptically from the intermediate auricular vein (*vena auricularis caudalis*) of the experimental (on the last day of experimental treatment) and control animals using sterile needles and heparinized plastic syringes. Three drops of blood were added to 4 ml of the complete blood cultivation medium PB Max Karyotyping (Gibco BRL). The blood cultures were incubated at 37 °C for 72 h. Colcemide at the final concentration of 0.6 $\mu g.ml^{-1}$ (Gibco BRL) was added 60 min prior to cell harvest. After the hypotonic treatment with 0.075 M potassium chloride solution (Gibco BRL) for 15 min and fixation in modified Carnoy's solution (3 : 1, methanol : acetic acid), the resuspended cells were placed onto frozen glass microslides, air-dried and stained for 6 min with 2 % Giemsa solution (Gibco BRL). Stained microslides were observed under the Leica light microscope. The chromosomal analysis was carried out using 25 c-metaphase spreads per rabbit taken from chromosome microphotographs (Parkanyi *et al*., 2004).

The $\chi^2$ test was used for statistical evaluation of the results.

## RESULTS AND DISCUSSION

The study was focused on examining a possible effect of amygdalin substance applied to rabbit at different concentrations and by different ways on the occurrence of aneuploidy. Proportion of the cells with diploid chromosome sets in the cell nuclei was ranged from 70.4 % in the P4 group to 82.4 % in the P1 group of rabbits (Table 1). The same occurrence of diploid cells (70.4 %) was found in the control group. Aneuploidy was represented exclusively by hypodiploid cells for experinental groups as well as for control group. The lowest percentage of aneuploidy was found in P3 rabbits (17.6 %), whilst the P4 group exhibited the highest

**Table 1: Evaluation of chromosomal number from c-metaphase spreads**

| Rabbit groups | Diploidy | Hypodiploidy | Polyploidy |
|---|---|---|---|
| Experimental | | | |
| P1 | 76.0 % (n = 95) | 21.6 % (n = 27) | 2.4 % (n = 3 ) |
| P2 | 72.8 % (n = 91) | 27.2 % (n = 34) | - |
| P3 | 82.4 % (n = 103) | 17.6 % (n = 22) | - |
| P4 | 70.4 % (n = 88) | 29.6 % (n = 37) | - |
| Control | | | |
| C | 70.4 % (n = 176) | 26.8 % (n = 67) | 2.8 % (n = 7) |

*n – the number of evaluated metaphase plates

presence of aneuploid nuclei (29.6 %) in comparison to control animals (26.8 %). According to the results of $\chi^2$ -test, no significant differences were found. Polyploid cells were found at low percentage in P1 (2.4 %) and control (2.8 %) groups.

Previous studies, performed specifically to reveal the rate of chromosome number variability, show that almost all tissues exhibit aneuploid cells (Iourov *et al.*, 2008). The incidence of chromosomal aneuploidies may depend on many factors, such as the source of cells, animal species, animal age, cell culture conditions and genetic manipulations (Czepulkowski, 2001). Aneuploidy in the present study was represented by hypodiploid cells ($2n < 44$). Average value of hypodiploid cells regardless to experimental groups was represented by 24 % (21.6, 27.2, 17.6 and 29.6, respectively). Control group exhibited 26.8 %. These relatively small differences among the groups may be explained by a random selection of evaluated nuclei. Higher proportion of hypodiploid cells (at average 41.47 % and 54.8 %, resp.) for non-treated rabbits have been recorded in the study of bone marrow cells, published by Curlej *et al*. (2007). Parkanyi *et al*. (2004) detected 38 % and 26 % presence of hypodiploid cells (average value 32 %) in the blood lymphocytes of control rabbits. Occurrence of cells with normal chromosome sets (diploid cells) in the present study is represented by average value of 75.4 % for experimental groups (measured values 76 %, 72.8 %, 82.4 %, and 70.4 %, resp.) in comparision to 70.4 % for control group.

Numbers of studies have been focused to reveal potential action of amygdalin substance to cell culture, especially those from cancer tissues. But still there is a lack of scientific records derived from the experiments with the model organisms about effect of amygdalin-derived substances on the structure or number of chromosomes in the cell nuclei. Studies by other authors suggest that the amygdalin in "safety" concentrations and admission way affect the viability of human cervical cancer HeLa cells (Chen *et al*., 2013). Such information brings a therapeutic option to use of this substance. Nevertheless, the anti-tumor mechanism of amygdalin is not completely clear (Song and Xu, 2014). Clinical trials and large retrospective studies showed some adverse reactions after large dose application, such as gastrointestinal tract reaction and headache (Barwina *et al*., 2013; Yang *et al.*, 2013; Karabulutlu, 2014). The ways of amygdalin application to rabbits in our study were chosen basing on the knowledge, that the toxicity of oral administration route is far greater than the intravenous route. The mean lethal dose (LD50) of amygdalin in rats was reported to be 880 $mg.kg^{-1}$ body weight by oral administration (Adewusi and Oke, 1985; Park *et al*., 2013). For the rabbits, mice and dogs the maximum tolerance dose for oral amygdalin intake has been published as 75 $mg.kg^{-1}$ (Zhang *et al*., 1986). We have decided for „safe" 60 $mg.kg^{-1}$ and risky 300 $mg.kg^{-1}$ doses. The LD50 of intravenous injection in mice is 25 $g.kg^{-1}$, while intra-peritoneal injection is 8 $g.kg^{-1}$. The maximum tolerance dose of intravenous and intramuscular injection of amygdalin in mice, rabbits and dogs is 3 $g.kg^{-1}$ (Rauws *et al*., 1982). On the basis of this records, intra-muscular doses 0.6 and 3 $mg.kg^{-1}$ used in our experiment may be considered as safe. The human's maximum tolerant dose of intravenous injection is approximately 70 $mg.kg^{-1}$ (Rauws *et al*., 1982). In human, systemic toxicity occurs after oral administration of 4 g amygdalin per day, lasted for half a month or intravenous injection of a month. If the dose is reduced to daily oral doses of 0.6 ~ 1g, it can avoid toxicity (Bromley *et al*., 2005; O'Brien *et al.*, 2005).

In conclusion, according to our results, amygdalin applied at chosen concentations orally or intramuscularly, showed no significant adverse effect represented by extra-creation of aneuploid cells in rabbits.

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