CONSIDERATIONS ON THE UTILITY OF *DIGITALIS LANATA* SPECIES FOR DRUG INDUSTRY

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Digitalis lanata has a well-deserved place in drug industry.This interest dates back in 1785, when the effects of Digitalis lanata extracts were discovered and digoxin became part of the heart failure treatment.

The xx century saw this interest growing as the drug industry expanded. The leaves of Digitalis lanata - the source of cardiotonic glycosides - are used in the treatment of heart failure as drug products only.

Digitalis leaves are rich in active principles (cardenolides or cardiotonic glycosides). Their chemical composition is complex besides the cardiac heterosydes being saponins and flavones, organic acids, lipids and glucids, tannins and mucilages. The digitalic biologicals (compositions) have a cardiotonic and diuretic action due to the glycosides from A group that increase the energy of cardiac contraction. The immediate effect is a much faster and complete heart drainage in the systole period, a better filling and reduction of the diastolic volume, a reduction of the venous pressure, and an increase of the mechanical efficiency of the myocardium. This fact allows the heart to fulfill its activity with minimum oxygen consumption. A further effect, due to the increase of the cardiac activity's efficiency is the increase of the urinary excretion. At present all the cardiotonic drugs are obtained industrially in our country only from the Digitalis lanata leaves. The results obtained with these species show that experimental mutagenesis can be an efficient method in obtaining some plants with superior bioproductive characteristics, insufficiently exploited in the amelioration activity of this type of plants. Nowadays mutagenesis is considered to be an indispensable way for obtaining some desired mutations and the mutations induction could be the unique alternative for the improvement of a character without affecting the rest of the genome. The intervention with mutagenic agents increases considerably the frequency of mutations appearance and if the number of mutations increases, the chance of some useful mutations identifications will be greater in amelioration. This work's objectives encompass the history of D. lanata, the current studies on its effects as well as its use in the perspective.

Key words: *Digitalis lanata, cardiotonic drugs digoxin, cardenolides.*

Digitalis lanata species is important for medicine industry. It has been studied even from the 1785 when *The Digitalis lanata* extract effects were discovered. We can talk about a chemistry of the digitalis leaves from 1824, when Paqui isolates some white crystals to which he gives '*Digitalin'* name and which presented an alkaline reaction.

In 1869 Nativelle obtained a crystallized composition named crystallized digitalin (*Digitaline Nativelle*), that for a long time represented the basis of the therapy with *Digitalis* in the Western European countries, and as a consequence Schneideberg isolated the same substance under the name "*Digitoxin*" [6, 19]. In 1930 Sydney Smith de la Wellcome isolates the glycosides of *Digitalis lanata*, and produces, starting from them, *Lanoxin* for the treatment of the cardiac insufficiency. Stoll and Kreis [15] precipitating the enzymes from the fresh leaves and succeed in isolating purpurea glycoside A and B; later they will separate those 3 genuine glycosides from *Digitalis lanata* and namely *lanatosides A,B* and *C*. In 1957 Renz obtains other two genuine glycosides, *lanatosides D* and *E*, and Smith isolates and examines the physical & chemical properties of the digoxin [19, 12] . The importance of this species for the pharmaceutical industry was established at the beginning of the 20th century when it has been found that *Digitalis lanata* leaves have got a rich content in cardiotonic heterosydes. This fact explains why today the most used vegetal material for the industrial extraction of the digitalic glycosides represents *Digitalis lanata* extracted from crops [6].

MATERIAL AND METOD

To highlight the importance of this species, for the pharmaceutical industry, at the achievement of this work, we used resources of literature. The literature that we have consulted were selected in order to emphasize in a more faithful manner, the role of this species between medicinal plants.

RESULTS AND DISCUSSION

Many researchers have been concerned with obtaining some more valuable species of Digitalis. *Digitalis* type has a very important role in the „in vitro" researches domain, the perspective of obtaining cardiotonic glycosides in unconventional ways being tempting. It has started from the premise that vegetative clonation on the vegetative way of some valuable individuals, by practicing meristems cultivation, could reduce the limits of mutability and could increase the chances of transmission (without segregation) of the suitable characters to the descendants. The capacity of producing cardenolides can be omitted during "in vitro" cultivation, reoccurring together with tissues organization. The regenerated tissues have presented the same type of synthesis the same as that of the generatively obtained plants. Thus, the genetic information for cardenolides production hasn't been altered during "in vitro" cultivations. Furuya, quoted by Kartnig, [14] showed that : regenerated *Digitalis* plants from tissues cultures that

didn't produce cardenolides keep their synthesis capacity, plants grown to maturity being capable of producing the same aceleaşi cardioglycosides.

The results obtained by Garve and his colaborators., [11]are different from those of Kartnig. They have studied the cardenolides synthesis in long term cultures of *Digitalis lanata*, that showed a reduced capacity or *unimportant* for "de novo" synthesis of the cardioglycosides, but they synthesized considerable quantities of saponins and other steroids. [4].The cultures were initiated from different organs of *Digitalis lanata*, the stromgest morphogenetic reaction being presented by the stem tissue and that of anthers [8] After the analysis there were discovered in both lines reduced quantities of cardenolides (equivalating with 0,1-1 mg/g s.u.). It has been noticed that both the hormonal composition of the culture medium and the level of morphological organization (globules, embrioides, primordies of sprouts and leaves) influence esentially the biosynthetic capacity of the new formed tissues.

The researches of Ohlsson and his colab. [17] Scheibner and his colab. [20] showed that reduced rates as a diminished digitoxine and chlorophyll content were obtained in yellow light or darkness. The digitoxine synthesis is influenced by the different spectral distribution. This stimulated the accumulation of cardenolides but not the chlorophyll (it was noticed that in general it's not a positive correlation between the 2 synthesis), yellow light (500-690 nm) didn't have any effect over the chlorophyll and digoxin synthesis. Scheibner,[20], showed that it's not an interdependence between the chlorophyll synthesis and cardenolides accumulation, fact confirmed by Hagimori hypothesis, [13], and Diettrich et al. (1988) sustain that the morphological diferentiation and not the chlorophyll formation or cloroplasts represent the essential factor for digitoxin synthesis in *Digitalis* cultures.

A series of researchers as: Diettrich, [7], Petersen, [18], Wendroth, [21], Kreis et al. [15], Alfermann et al. [1], have tried to elucidate the enzymes action in the biosynthesis of the secondary metabolites in the cellular cultures of *Digitalis*, and also of the enzymes that interfere in the bioconversion of the cardiotonic glycosides. It has been given a special attention to the reaction of 1-2-3-hidroxylars, reaction that has made profitable the cellular cultures of *Digitalis lanata* and *Digitalis purpurea*.

The constant concern of improving the process of biosynthesis and that of increasing the output accumulation of cardiotonic glycosides was also stimulated by the possibility of transformation with *Agrobacterium sp*. of the roots (hairy roots) with plant elicitors. [2]

At *Digitalis lanata* there were elaborated methods that allow transformation with *Agrobacterium tumefaciens*, using plasmids of wild type and also plasmids genetically manipulated with bacteria marker and receptor genes.The best results were obtained after infecting the cultures with *Agrobacterium rizogenes* and with *Agrobacterium tumefaciens*, that determines a strong ramification and proliferation of the tissues. (hairy roots). The transformed roots have a particular morphology, tap abundantly and are covered by absorbent hairs [16]. The contamination has as

effect the stimulation of the biosynthesis capacity and a rapid and constant growth of the quantity of secondary metabolites. [4,5]

The tumours (nodosities) were obtained by trasforming the leaves and sprouts of *Digitalis lanata* with different stems of *Agrobacterium tumefaciens*. The formed tumours on *Digitalis lanata* leaves contain ‚digitoxigeninic' derivates.

The transformed cells of *Digitalis lanata* have the capacity of synthesizing "de novo", the thing that can't be done in cellular cultures "normal" at this species. A more recent method of increasing the production and of the secondary metabolites accumulation in cellular cultures is the induction in them of the chemical reactions of protection-elicitation. [9,2]

The elicitation of an embriogenic stem of *Digitalis lanata* was done by the replacement of 2,4-D from the medium (that favored the rapid development of some parenchimatose cells) with NAA and the reduction of the, rate in the culture environment. In culture optimum conditions the total of accumulated cardenolides was bigger after a period of cultivation of 30 days [20]

At *Digitalis lanata* tetraploide were obtained (by means of colchicine) with a reduced content of cardenolides, but raised of *lanatozida A* unlike the diploid form. At this species Michalski, (1963), quoted by Floria, obtained valuable mutants by gamma iradiation (15-20 kR), with an increased content of cardenolides, but that presented stems, leaves and flowers modifications. At present time mutagenesis is considered as being an indispensable way for obtaining some desired mutations, and the induction of the mutations could be the only alternative for the improvement of a character without affecting the rest of the genoms. The intervention with mutagenic agents increases the frequency considerably of mutations appearance and the bigger the mutations number the bigger the chance of identification of some useful mutations in improvement. The digitalis with fast action are indicated in left ventricular insufficiency (severe pulmonary edema) and especially in paroxistic and rapid atrial arrhythmias**,** badly tollerated.The administered digitalis in therapeutic dosis The digitalis administered in therapeutic dosis increases the contraction power of the myocardium**,** rarefying and regularizing heart beatings rhythms**.** At the same time the amplitude of these beatings increases. Blood pressure is normalized at the same time with amelioration of the cardiac insufficiency.

Digitalis leaves also have a diuretic action due to the saponosides and flavones presence in their composition. Lately, the Japanese researchers have reached the conclusion that some cardiotonics like digitoxine, can be used in the treatment of some skin tumours or of mice lungs**,** so the use of these active principles could be extended even in the treatment of other diseases**,** different from those of the myocardium. The digitalis represent biological remedies compatible with human nature, because their chemical structure is similar to sterolic structures sterolice proper to the human body.

CONCLUSIONS

The researches regarding this species are very numerous and highlight the importance of this species, for the pharmaceutical industry

Digitalis leaves are rich in active principles (cardenolides or cardiotonic glycosides). Their chemical composition is complex besides the cardiac heterosydes being saponins and flavones, organic acids, lipids and glucids, tannins and mucilages.

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