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# EFFECT OF ISOFLAVONE TREATMENT ON SOLID EHRlich TUMOR GROWTH

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## ABSTRACT

*Epidemiological studies reveal that the regular consumption of soybean reduces or suppresses breast cancer, being this fact associated to the presence of isoflavone, phytoestrogens in soybeans. It is believed estrogen that enables the growth of human mammary cancer and, consequently, the use of phytoestrogen has been considered to be an alternative therapy to the use of estrogen. In this study, the action of the isoflavones, a phytoestrogen that can be found in soybean, was evaluated in the growth of the Ehrlich tumor. This evaluation was based on the determination of weight, area and cellular proliferation. For that, an isoflavone suspension was given, via ip, in a dose of 20 mg/kg of weight, for 20 days. It was observed that the treatment can interfere with the cellular division cycle, based on the fact that the treatment with the isoflavone suspension reduced the number of mitosis found in the tumor tissue. However, no significant differences in weight and dimensions of tumor mass between the treated group and the control group were found, and that the necrosis area extension might be influencing these parameters, fact that deserves future investigation.*

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## INTRODUCTION

Breast adenocarcinoma is one of the most recurrent cancers in humans. The growth of this tumor is influenced by strogen, whose role is to induce mitoses in primed cells (EHRlich, P; APOLANT, H., 1905; LEE, D. S. et al., 2001).

There is evidence that this female hormone interferes in the expression of the bcl-23 gene that controls the apoptosis process. It is known that in tumor cells, apoptosis, or scheduled cell death, is delayed. Such effect of strogen in relation to the tumor growth has been found to be dependent on its concentration (CUEVAS, J.; SIRBASKU, D., 2000; SUH, K. S., 2003).

Therefore, the use of strogen in hormonal reposition therapy was challenged. A marked advance in this field was the availability of a natural component of soybean: genistein, an isoflavone agonist of the strogen, which started to be used sucessfully in hormonal reposition therapy without showing the side effects associated to strogens (MESSINA, M. J., 1994).

Studies with this flavonoid revealed their antitumoral effect when associated to specific antibodies in the treatment of lymphoid leukemia (UPADHYAY, S. et al., 2001). The use of genistein, in association with antineoplastic drugs, improves the rate of cell maturation and inhibits the proliferation of leukemic cells (LOWENTHAL, H.; JAHN, G., 1932).

In studies on tumors whose growth is influenced by hormones, such as the breast and prostate tumors, genistein showed important chemopreventive effect (THIS et al., 2001), and was found to be able to reduce the incidence of metastasis in advanced phase tumors (SHAO, Z. M. et al., 1998). In bladder neoplasias the treatment with isoflavone was effective through a combination of direct effects in the tumor cells and indirect ones in the angiogenesis of the tumor (ZAVA, D. T.; DUWE, G., 1997).

The mechanism of action of genistein is related to its affinity to b receptors in the breast glandular epithelium, whereas strogen links to two types of receptors – a and b –, and that such mechanism can lead to protection against breast cancer due to the action in the proliferation and differentiation that it has in the mamarian gland (CONTRONEO, M. S. et al., 2002). It is believed that genistein reduces the circulating level of ovarian hormone in premenopause women and that lower levels of this hormone can

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reduce the proliferation of breast cells and reduce the risk for breast cancer (MAZZUR, W.; ADLERCREUTZ, H., 1998).

In this study the effect of isoflavones in the growth of Ehrlich solid tumor (GOODMAN, G. et al., 1996; LU, L. et al., 2000), a murine tumor originally mamarian, transplanted with success to the subcutaneous tissue is evaluated.

## MATERIALS AND METHODS

Swiss male mice with age ranging from 30 to 45 days from the biotery of the University of the Sacred Heart – USC – Bauru, were used in this experiment.

Ehrlich tumor was used and the neoplastic cells kept *in vivo* in weekly transplant in the mice through implantation of  $10^7$  tumor cells in the sub-cutaneous. To obtain tumor implant inoculum the ascetic fluid was harvested from the peritoneal cavity and the suspension was washed in buffered antipyrogenic saline solution (1500 rpm, 10 minutes) and adjusted to  $10^8$  cell/mL .

To investigate the cell viability it was used the exclusion test by the Tripan blue. In all protocols only the suspensions with viability greater than 95% were used. Isoflavone was administered daily via intraperitoneal, in doses of 20 mg/kg<sup>6</sup> for 20 days.

The tumors were removed from animals, fixed and processed routinely for HE. The assessment of the tumoral growth was done by *weighing the tumor* in an analytical scale, *measuring the tumoral mass* with a pachymeter taking as reference the dimension of the greater length of the tumor, *counting the number of mitoses* in slides stained by HE and studied in a microscope.

The non-parametric test of Mann-Whitney was used and the results were discussed at a level of 5% of significance.

## RESULTS

It was observed that in the experimental conditions there were no significant variations in the tumor weight between the treated and the control groups (TABLE 1).

TABLE 1 – Effect of treatment with isoflavone in the weight of the Ehrlich solid tumor.

	Treatment		
	Isoflavone	Control	Statistic test
Weight	0.25 $\bar{\pm}$ 0.05* (0.10; 0.60)**	0.30 $\bar{\pm}$ 0.35 (0.05; 1.10)	0.67** *

\* – Median interquartile semi-amplitude.

\*\* – Minimum and maximum values.

\*\*\* –  $P > 0.05$ .

In the same way, in relation to the tumor area, no difference was found between the dimension of the tumor mass when comparing the treated and control groups. Data can be seen in TABLE 2.

TABLE 2 – Effect of treatment with isoflavone in the dimensions of the Ehrlich solid tumor.

Variable	Treatment		
	Isoflavone	Control	Statistic test
D maximum	1.00 $\bar{\pm}$ 0.16* (0.60; 3.00)**	1.20 $\bar{\pm}$ 0.50 (0.60; 2.00)	0.44** *
D minimum	0.75 $\bar{\pm}$ 0.11 (0.50; 1.00)	0.90 $\bar{\pm}$ 0.30 (0.40; 1.90)	1.55
Area	0.64 $\bar{\pm}$ 0.17 (0.28; 1.41)	0.85 $\bar{\pm}$ 0.53 (0.19; 2.69)	0.96

\* – Median interquartile semi-amplitude.

\*\* – Minimum and maximum values.

\*\*\* –  $P > 0.05$ .

The treatment with isoflavone affected significantly the number of mitoses in the tumoral parenchyma. The treated group showed the least cells in mitotic phase than the number of mitoses found in the cell of the tumor of the control group, as can be seen in TABLE 3. This fact suggests that the treatment with isoflavone has interfered in the cell cycle, affecting the tumoral growth as per the model used in the present study.

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TABLE 3 – Effect of the treatment with isoflavone in the number of mitoses present in the tumoral tissue.

	Treatment		
	Isoflavone	Control	Statistical test
Number of mitoses	8.50 $\bar{\pm}$ 1.50* (5.00; 14.00)* *	17.00 $\bar{\pm}$ 1.50 (5.00; 20.00)	3.96***

\* – Median  $\pm$  semiamplitude interquartilic.

\*\* – Minimum and maximum values.

\*\*\* –  $P < 0.0001$ .

## DISCUSSION

The treatment with genistein, one of the components of isoflavone, inhibits the proliferation of positive and negative estrogenic receptors in breast cancer cell lineages characterized, among other things, by the specific restrain of G2/M phase of the cell cycle (CONSTANTINO, 1998), accompanied by decrease in the number of cells in the phases G1 and S (SAHO, 1998). In the present study the treatment with isoflavone has confirmed the inhibitory capacity of the tumoral growth by its amniotic property.

The tumoral weight and dimensions were not altered; however, the cell types were found in different proportions, that is, there was a greater number of cell in mitoses in the control group than in the treated group, a fact probably due to the blocking of the progression of the cell cycle and possibly associated to the activation of the p21<sup>waf1</sup> gene which, together with the inactivation of the bcl-2 gene, induce to apoptosis (CONSTANTINO, 1998, UPADHYAY, 2001). Apoptosis is the common way by which all tumoral cells treated with genistein are destroyed, restraining thus the tumoral growth (ZHOU, 1998, CONSTANTINO, 1998, UPADHYAY, 2001, SUH, 2003).

The decrease of viable cells, as observed in mice tumors of the treated group and associated to the apoptotic phenomenon, in metastatic neoplasias, reduces the incidence of metastasis. According to Setchell (1998), genistein can reduce the metastatic dissemination of tumors in advanced phase, as is the case of bladder tumor of rats in which Zhou (1998) verified that the soya isoflavones, besides the potential to inhibit the tumor growth by apoptotic phenomenon, was able to restrain angiogenesis, that is, restrain the proliferation of tumor cell and the neovascularity of the tumor.

Another property of genistein as reported by Conklin (2000) seems to guarantee the inhibition of the tumor growth: the inactivation of enzymes: (i) tyrosine kinase, responsible for cell protection against apoptosis (NEDLJKOVIAE, 2001, CLARCK,1996) and (ii) topoisomerase II, essential to the cancer cell replication, specially those of breast cancer (CONKLIN,2000). The tumoral model used in this study deals originally with a breast and spontaneous tumor that, being transplanted to the peritoneal cavity, has adapted by bluming some of its original genetic characteristics; however, it can possibly maintain some of its primary characteristics. In this way, it is also possible to credit the antiproliferative activity of tumor cells by genistein to these two properties described before. Another fact to be stressed is that, in the treated group, the necrosis area seems to be more extensive than that of the tumors in the control group. This leads to the need of evaluation of the necrosis area in further studies to assess its statistical significance.

Even tough the effect of isoflavone was not assessed in females in the present study, further research is needed to clarify the capacity of genistein to compete with strogen for strogenic receptors.

## CONCLUSION

The treatment with isoflavone had influence in the growth of Ehrlich solid tumor. This can be proved by the small number of mitoses present in the tumor tissue when animals were injected with the isoflavone suspension.

Further studies are needed to quantify the necrosis area and its influence in tumor weight and dimension, since the present study, despite the reduced number of mitoses in the treated group, did not detect differences in these parameters.

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