

# Synthetic phosphoethanolamine: the state of the art of scientific production

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Cancer is a multifactorial disease and a serious public health problem. Currently, alternative drug treatments for cancer are actively being sought, which is the case of synthetic phosphoethanolamine (PHOS-S), a compound that could possibly have anticarcinogenic effects. To analyze the available scientific evidence to evaluate the anticarcinogenic effects of *in vivo* and *in vitro* PHOS-S. A systematic literature review of scientific articles aimed at evaluating the anticarcinogenic potential of PHOS-S, *in vivo* and *in vitro*, using the databases PubMed, ScienceDirect, SciElo, CAPES Portal and LILACS. The selected papers suggest a possible anticarcinogenic effect of PHOS-S by inhibiting tumor growth by inducing apoptosis and cell cycle blockade as well as cytotoxic potential against leukemia cells. However, a possible stimulatory effect of tumor growth was also observed. Although some of the evaluated studies indicated a possible anticarcinogenic effect of PHOS-S, the limitations of these studies must be evaluated. Most were performed by the same research group, and in the scientific literature, we identified only preclinical studies (in cells or in animals). No human study has been published. Thus, more studies are needed to confirm the anticarcinogenic capacity of PHOS-S.

**Keywords:** Synthetic phosphoethanolamine/effects/*in vivo/in vitro*. Antineoplastic Agent. Cancer.

## INTRODUCTION

Cancer has become a serious public health problem in both developed and developing countries. Cancer is the second leading cause of death globally and accounted for 8.8 million deaths in 2015 (WHO, 2017). Current data and statistical projections indicate that, in 2016, 1,685,210 new cancer cases and 595,690 cancer deaths will occur in the United States alone (Siegel, Miller, Jemal, 2016).

Cancer must be understood as a multifactorial disease caused by a confluence of both endogenous and environmental factors. Diet and food, life habits and the environment in which a person lives may contribute to an increase in the likelihood of being affected by cancer. For example, the increased consumption of foods rich in food additives, alcohol consumption or smoking induce the emergence of cancer. Therefore, the incidence of cancer has been increasing steadily, and an increasing number of

individuals are receiving cancer treatments (Boyle, Levin, 2008; Doll, Peto, 1981; Anastácio *et al.*, 2016).

Currently, the standard cancer treatment is based on surgery, chemotherapy and radiation therapy, depending on the type and characteristics of the tumor (WHO, 2002; Weinberg, 2014). However, this type of conservative treatment can generate various types of side effects that decrease the quality of life of patients and may also hamper treatment. Furthermore, many cancers remain uncured even after therapy (WHO, 2002; Sikora *et al.*, 1999; Macquart-Moulin, 1997).

Such outcomes have inspired modern medicine to search for safer and more effective chemotherapy drugs that act only at the specific local site of cancer cells (Gullotti, Yeo, 2010) or new therapeutic alternatives for the treatment of cancer (Vidal, Carvalho, Bispo, 2013). These alternative treatments may be based, for example, on changing the eating habits of the patient (Yu *et al.*, 2009) or adopting new, non-conventional drugs for clinical treatment. An example of the latter is synthetic phosphoethanolamine (PHOS-S), a compound that has been gaining attention as a possible anticarcinogenic

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agent (Pondé, Ades, Azambuja, 2016; Ferreira, 2013; Meneguelo, 2007).

Phosphoethanolamine was first isolated in 1936 by Outhouse (Outhouse, 1936). The phospholipid ethanolamine plays diverse cellular roles, including in cell division and apoptosis, and phosphoethanolamine is a primary amine that makes up the cell membranes of many tissues (Brasil, 2013; Bakovic, Fullerton, Michel, 2007). This compound has been studied as a possible anticancer agent in response to evidence that it can suppress tumor growth (Ferreira, 2013; Meneguelo, 2007; Veronez, 2012).

However, this compound has not yet received approval for use as an anticancer drug. PHOS-S has just begun its clinical trials in humans, so, the analyses of its anticancer proprieties are based solely on laboratory models, resulting in uncertainties about the use of PHOS-S (Sarraf *et al.*, 2016; Pivetta 2016).

Therefore, this systematic review aimed to analyze in detail the available scientific evidence to evaluate the anticarcinogenic effects of PHOS-S both in animals and *in vitro*.

## METHODOLOGY

This systematic review was guided by the recommendations established by the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Moher *et al.*, 2009). Five databases were used: PubMed, ScienceDirect, CAPES Portal, SciELO and LILACS. The databases were searched for articles evaluating the anticarcinogenic potential of PHOS-S.

The following terms were used as descriptors (previously consulted MeSH – Medical Subject Headings): neoplasm and cancer. Since the term synthetic phosphoethanolamine is not a descriptor, it was used as a keyword in the search. To perform the search, the authors used the descriptor “synthetic phosphoethanolamine” with the Boolean operator AND, followed by one of the two descriptors, ‘neoplasm’ or ‘cancer’. According to the guiding question was established “PICOS”: “P” (problem): the anticarcinogenic effects of PHOS-S; “I” (intervention): PHOS-S; “C” (Comparison): control (untreated); “O” (outcomes): tumor regression; “S” (study design): Pre-clinical trials (in cells or in animals), because there is no clinical studies of PHOS-S published in scientific articles.

Thirty-six articles were found. Duplicate articles with a later date of publication were excluded, and the authors applied eligibility criteria (inclusion and exclusion criteria) to the remaining articles to determine which

publications to use in this review by analyzing the type of article (original and review - selected only original articles) and the abstract. These criteria were checked independently in a blinded manner by two authors of the present review who were provided general information and abstracts of the articles. After the selection of the articles, the following variables were collected: author, year of publication, country of study, animals used in *in vivo* tests, cell lines used for *in vitro* studies, sample size and doses (*in vivo* studies), objective of the study and main result.

## RESULTS

Eight papers were selected as the basis for this review, as is presented in the flow diagram, shown in Figure 1, that summarizes the methodology of the article. After analysis, the authors concluded that all eight articles met the requirements for inclusion.

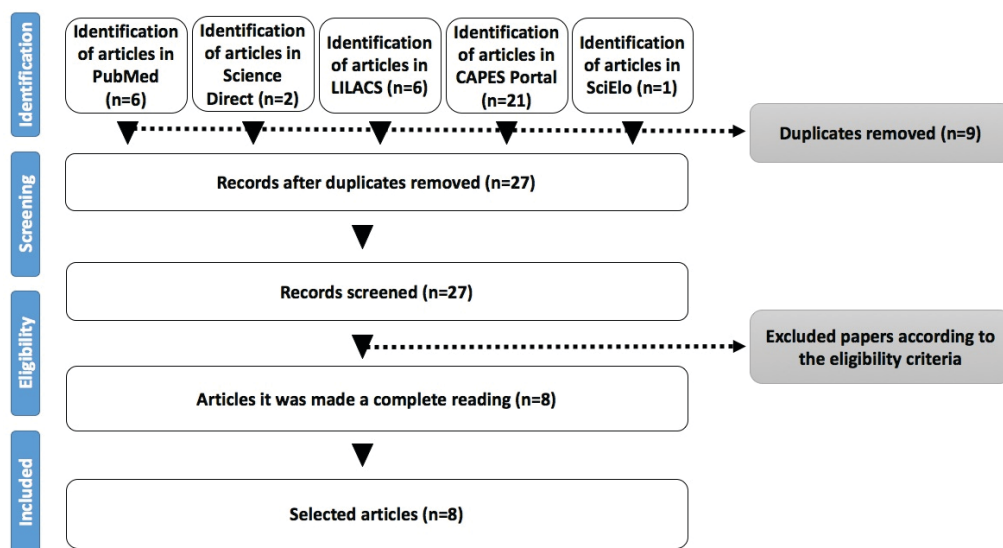
Eight articles were analyzed to obtain the results of the presented review. The general information of the selected articles is presented in Tables I and II.

Several studies of PHOS-S have been performed. This substance has been linked to anticarcinogenic effects and reportedly alters the cell metabolism of the immune response (Arruda *et al.*, 2011), elicits anti-tumor effects (Ferreira *et al.*, 2011, 2012a,b, 2013a,b), induces apoptosis in cells (Ferreira *et al.*, 2011, 2012a,b, 2013a,b), elicits antileukemic effects (Ferreira *et al.*, 2013c), possesses antiangiogenic (Ferreira *et al.*, 2013b) and antimetastatic activity (Ferreira *et al.*, 2011, 2013b), and, conversely, stimulates tumor proliferation (Kano-Sueoka *et al.*, 1979).

### Alterations in the cells of the immune response

The ability of PHOS-S to modulate the metabolic activity of macrophages, components of the immune system, has been evaluated. These experiments were performed to assess the ability of the compound to inhibit tumor growth by altering the activity of these cells (Arruda *et al.*, 2011).

In laboratory tests, Ehrlich ascites tumor cell lines were inoculated in mice fed diets containing 800 mg/kg PHOS-S. The researchers surgically removed the inoculated tumors (after a period of growth) and washed the abdominal cavity to remove peritoneal macrophages. The macrophages were then subjected to the following assays: spreading test and calculation of H<sub>2</sub>O<sub>2</sub> and NO (substances involved in tumor progression and dispersion) (Arruda *et al.*, 2011).



**FIGURE 1** - Flow diagram of search procedure and screening process through the different phases of the systematic review.

These analyses indicated that PHOS-S affects the metabolism of macrophages; cells obtained from mice fed PHOS-S showed less dispersion in the spreading test and released minor amounts of  $H_2O_2$  and NO. Moreover, the histopathology of the excised tumors showed greater cell cohesion of tumor cells retrieved from mice treated with PHOS-S (Arruda *et al.*, 2011).

### Antitumor effects

To verify a possible anticancer action, PHOS-S was tested in various tumor types (Ehrlich, melanomas, breast adenocarcinoma, renal carcinoma and mucoepidermoid pulmonary carcinoma) to ascertain its antitumor capacity (Ferreira *et al.*, 2011, 2012a,b; 2013b).

The antitumor capacity of PHOS-S was evaluated in Ehrlich ascites tumor cells and other tumor cell lines (human breast adenocarcinoma [MCF-7], human malignant melanoma [SKMEL-28 and MeWo] murine malignant melanoma [B16-F10] and human mucoepidermoid pulmonary carcinoma [H292]). The researchers confirmed by MTT colorimetric assay (0.5 to 10 mg/mL) and Hoechst 33342/PI fluorescence assay (2.30 mg/mL) that PHOS-S has *in vitro* cytotoxic activity against tumor cells while preserving healthy cells and can cause morphological alterations in tumor lines. PHOS-S also directly inhibited tumor growth in *in vivo* models (at doses of 35 and 70 mg/kg/day). Finally, PHOS-S also decreased tumor proliferation and altered the cell cycle in these cells (reducing the proportion of cells in G2/M phase and increasing the number of apoptotic sub-G1 cells) (Ferreira *et al.*, 2012a).

Other studies analyzed the ability of PHOS-S to inhibit the growth of melanoma in murine models (Ferreira *et al.*, 2011; 2012b). In one of these studies, groups of mice with melanomas (originating from B16F10 melanoma strain cells) received PHOS-S solutions (7 mg/kg and 14 mg/kg) via injection into the respective tumors. This particular study also studied the antitumor capacity of PHOS-S in tumor cells and healthy cells (*in vitro*) exposed to various concentrations of PHOS-S (0.005 to 6.0 mg/mL) (Ferreira *et al.*, 2011).

In both experiments, PHOS-S exhibited antitumor effects. In the *in vivo* models, PHOS-S attained 86% tumor reduction, along with an increase in the apoptosis rate in the cells of tumors treated with PHOS-S. In the *in vitro* models, PHOS-S exhibited cytotoxic activity in tumor cells and decreased the mitotic index of this cell population (Ferreira *et al.*, 2011).

Another study analyzed the antiproliferative capacity of PHOS-S in melanoma B16F10 tumor cells in both *in vivo* and *in vitro* models. When examined *in vitro*, PHOS-S showed antiproliferative capacity in an MTT colorimetric assay (12.5 to 100 mM without inducing morphological changes of the tumor cells. Additionally, in *in vivo* models, PHOS-S (50 and 100 mg/kg) showed antiproliferative capacity, decreasing the tumors size (by 60% and 47%, respectively), delaying tumor growth and significantly enhancing mouse survival (Ferreira *et al.*, 2012b).

The ability of PHOS-S to inhibit tumor growth of MCF-7 breast cancer cell lines has also been reported. In this study, researchers ascertained that the compound had cytotoxic capacity based on the results of MTT

**TABLE I** - Description of the articles included in the systematic review of the synthetic phosphoethanolamine and its anticarcinogenic effects

Author & Year	Sample size of <i>in vivo</i> tests and tested concentrations of synthetic phosphoethanolamine (PHO-S)	Main Conclusions
Kano-Sueoka <i>et al.</i> (1979)	Not applicable	Phosphoethanolamine induced tumor proliferation
Arruda <i>et al.</i> (2011)	n=20, group treated with 800 mg/kg PHOS-S; n=20, control group	PHOS-S has the capacity to alter macrophage metabolism
Ferreira <i>et al.</i> (2011)	30 mice (divided into 4 treatment groups: 7 mg/kg PHOS-S, 14 mg/kg PHOS-S, 7 mg/kg Taxol and saline control)	PHOS-S showed anticarcinogenic capacity, inhibiting tumor growth by induction of apoptosis
Ferreira <i>et al.</i> (2012a)	The mice were divided into different groups (n=5): 35 mg/kg/day PHOS-S, 70 mg/kg/day PHOS-S and an untreated control group	PHO-S presented cytotoxic potential against tumor cells via several mechanisms
Ferreira <i>et al.</i> (2012b)	The mice were divided into four groups (n=5): 50 mg/kg PHOS-S, 100mg/kg PHOS-S and an untreated control group	PHOS-S showed inhibitory capacity in B16F10 cells
Ferreira <i>et al.</i> (2013a)	Not applicable	PHOS-S presented anticarcinogenic capacity, inhibiting tumor growth by inducing apoptosis and cell cycle blockade
Ferreira <i>et al.</i> (2013c)	The mice were divided into different groups (n=5): 40 mg/kg PHOS-S; 80 mg/kg PHOS-S; 1 mg/kg all-trans retinoic acid (ATRA) and 10 mg/kg daunorubicin (DA) (the last two were positive controls). In addition, mice were treated with 100 mL of PBS (vehicle) to induce the same stress conditions of treatment in the control animals	PHOS-S presented cytotoxic capacity against leukemic cells
Ferreira <i>et al.</i> (2013b)	The mice were divided into different groups (n=5): 50 mg/kg/day PHOS-S, 100 mg/kg/day PHOS-S and 10 mg/kg/day sunitinib	PHOS-S presented antiangiogenic and antimetastatic capacity

colorimetric assays (from 20 mM) and reduced tumor cell metabolism (Ferreira *et al.*, 2013a).

Finally, PHOS-S exhibited cytotoxic effects on murine renal carcinoma cells. Based on the results of MTT colorimetric assays, the researchers concluded that PHOS-S has concentration-dependent cytotoxic activity against tumor cells. In addition, the IC<sub>50</sub> value (half the maximum inhibitory concentration) of PHOS-S in these cells was 90 mM (Ferreira *et al.*, 2013b).

### Apoptosis induction effects

Based on the notion that phospholipids may be related to the induction of apoptosis (Meneguelo, 2007; Veronez, 2012; Elbayoumi *et al.*, 2016), the ability of PHOS-S to induce apoptosis in cancer cells has been examined.

Studies in B16-F10 melanoma and MCF-7 breast cancer cell lines indicated that PHOS-S can induce tumor cell apoptosis regardless of blocking of the Bcl-2 protein. Loss of Bcl-2 expression was observed in tumor cells treated with the compound (Ferreira *et al.*, 2011, 2013a).

Several studies have observed that PHOS-S can change the mitochondrial membrane potential, based on assays with Rhodamine-123 (Rho123). In these studies, PHOS-S reduced the membrane potential of mitochondria and caused dysfunction of these organelles in tumor cells (B16-F10 melanoma, Ehrlich ascites tumor, breast cancer MCF-7) pretreated with PHOS-S. These changes lead to an increased rate of apoptosis (Ferreira *et al.*, 2012a,b, 2012b., 2013a).

The influence of PHOS-S on caspase-3 activity has also been studied by flow cytometry in tumor cells (B16-F10 melanoma, Ehrlich ascites tumor, MCF-7 breast

**TABLE II** - Description of the articles included in the systematic review of the synthetic phosphoethanolamine and its anticarcinogenic effects, according to the main goals

Author & Year	Main Goal	Animal / Cell Lines Used For Study	Country
Kano-Sueoka <i>et al.</i> (1979)	To evaluate the possible role of phosphoethanolamine as a growth factor in breast carcinoma	MCCLX (64-24 cells) cell lines	United States
Arruda <i>et al.</i> (2011)	To examine the effects of a diet rich in synthetic phosphoethanolamine (PHOS-S) and its effects on the metabolism of macrophages and subsequent tumor development	Swiss male mice Ehrlich ascites tumor	Brazil
Ferreira <i>et al.</i> (2011)	To evaluate the anticarcinogenic effects of PHOS-S, <i>in vitro</i> and <i>in vivo</i> , using melanoma cells (B16-F10) and normal human fibroblasts	BALB-c female mice B16F10 cell lines	Brazil
Ferreira <i>et al.</i> (2012a)	To evaluate the anticarcinogenic effects of PHO-S in tumor cells	BALB-c Mice Cell lines: MCF-7, Skmel-28, Mewo, B16F10, H292 and Ehrlich ascites tumor	Brazil
Ferreira <i>et al.</i> (2012b)	To investigate the anticarcinogenic potential of PHO-S in melanoma B16-F10 cells both <i>in vivo</i> and <i>in vitro</i>	C57BL/6 J Mice B16-F10 cell lines	Brazil
Ferreira <i>et al.</i> (2013a)	To evaluate the anticarcinogenic activity of PHO-S in MCF-7 breast cancer cells	MCF-7 cell lines	Brazil
Ferreira <i>et al.</i> (2013c)	To investigate the antileukemic effects of PHO-S using acute promyelocytic leukemia (APL) as a model	NOD/SCID Mice KG-1, K562 and Jurkat cell lines	Brazil
Ferreira <i>et al.</i> (2013b)	To assess the anti-angiogenic and antimetastatic activity of PHO-S	HUVEC - CRL 1730 cell lines	Brazil

cancer) treated with PHOS-S. An increase in caspase-3 activity was observed, confirming that PHOS-S can control tumor development by inducing apoptosis via the caspase pathway (Ferreira *et al.*, 2011;2012a,b; 2013a,b).

Other proteins analyzed in studies of PHOS-S include cyclin D1 and p53. Flow cytometry revealed that PHOS-S increased p53 expression and suppressed the expression of cyclin D1. Therefore, the authors hypothesized that PHOS-S may trigger apoptosis and have antitumor potential (Ferreira *et al.*, 2012b, 2013b).

Finally, the included studies also analyzed the potential of PHOS-S to induce apoptosis by Annexin V/PI

double-staining and flow cytometry. The authors concluded that PHOS-S can induce tumor cell apoptosis, as tumors treated with PHOS-S had greater numbers of cells at the early and late stages of apoptosis. PHOS-S also induced morphological changes in the tumor cells. Based on these findings, the authors hypothesized a role of PHOS-S in inducing apoptosis (Ferreira *et al.*, 2011, 2012a).

### Antileukemic effects

A study analyzed whether PHOS-S possesses antileukemic effects. Leukemia cell lines (KG-1, K562



and Jurkat) were exposed to PHOS-S in different types of experiments (Ferreira *et al.*, 2013c).

The MTT colorimetric assay revealed cytotoxic effects of PHOS-S in the cell lines studied. PHOS-S also exhibited potent dose-dependent cytotoxic effects against KG-1, K562 and Jurkat cells, with IC<sub>50</sub> values of 9, 6 and 12 mM, respectively (Ferreira *et al.*, 2013c).

Flow cytometry also revealed that, *in vitro*, PHOS-S can decrease the mitochondrial membrane potential in the leukemic cell lines studied. Furthermore, the activity of caspase-3 in these cells was analyzed by the Annexin-V/PI double-staining apoptosis detection method. An increase in protein activity was observed in the studied cell lines, consistent with the evidence of the induction of apoptosis via a mitochondrial-dependent pathway by PHOS-S (Ferreira *et al.*, 2013c).

The researchers also analyzed leukemic models *in vivo* (acute promyelocytic leukemia) obtained from leukemic induction in mice (mice NOD/SCID). In these leukemic mice, cyto-morphological analysis revealed a smaller number of immature cells in the bone marrow, peripheral blood, spleen and liver (tested at concentrations of 40 or 80 mg/kg PHOS-S) (Ferreira *et al.*, 2013c).

Moreover, reductions of CD117<sup>+</sup> and Gr-1<sup>+</sup> cells (commonly observed in acute promyelocytic leukemia) were observed in the previously analyzed organs at the tested concentrations of the compound (40 or 80 mg/kg). Treatment with 40 mg/kg induced an increase in the percentage of CD45<sup>+</sup> cells, a decrease in hematopoietic progenitor cells from CD34<sup>+</sup> and a decrease in CD34<sup>+</sup> cells in the bone marrow of mice (Ferreira *et al.*, 2013c).

Finally, researchers also evaluated the effects of PHOS-S on the induction of apoptosis by Annexin-V/PI double-staining of CD117<sup>+</sup> and Gr-1<sup>+</sup> cells. The researchers observed an increase in apoptosis in the cells studied in the liver and spleen of guinea pigs pretreated with PHOS-S. These findings corroborate the possible antileukemic effects of synthetic phosphoethanolamine (Ferreira *et al.*, 2013c).

### Antiangiogenic activity

The ability of PHOS-S to influence the migration of endothelial cells was analyzed by wound healing assays. The researchers concluded that PHOS-S (10 mM) inhibited cell migration compared to the controls. In addition, human umbilical vein endothelial cells (HUVEC) treated with PHOS-S exhibited inhibition of the formation of capillaries in the tube formation assay, a three-dimensional model, confirming the antiangiogenic activity of PHOS-S (10 mM) (Ferreira *et al.*, 2013b).

PHOS-S also inhibited the mRNA expression of cyclin D1 and VEGFR1 (vascular endothelial growth factor receptor 1). These factors are involved in angiogenesis modulation from cell proliferation and induction of the angiogenic mechanism and were suppressed by PHOS-S, consequently reducing angiogenesis. PHOS-S also inhibited oxidative stress in murine renal carcinoma cells, reducing the formation of malondialdehyde and H<sub>2</sub>O<sub>2</sub> (90 mM) and therefore exerting a protective effect (Ferreira *et al.*, 2013b).

### Anti-metastatic activity

The anti-metastatic activity of PHOS-S has also been evaluated. Murine renal carcinoma cells were injected into mice (BALB). After 15 days of treatment (intraperitoneal administration of PHOS-S, 50 or 100 mg/kg/day), metastatic nodules were examined in the lungs and other organs of the animals (Ferreira *et al.*, 2013b).

The number of metastases in the lungs and spreading to other organs was lower in mice that received prior administration of PHOS-S. Furthermore, the cell density of metastatic nodules in treated mice was lower than that in the control mice in the experiment, suggesting anti-metastatic properties of PHOS-S (Ferreira *et al.*, 2013b).

In another study, mice with melanoma (B16-F10) tumors were treated with different doses of PHOS-S (7 and 14 mg/kg). The control mice exhibited a higher number of metastases compared with the treated mice, again confirming the anti-metastatic potential of PHOS-S (Ferreira *et al.*, 2011).

### Effects on the promotion of tumor cell proliferation

Researchers evaluated the possible role of PHOS-S as an inducer of tumor proliferation in a murine mammary carcinoma line (64-24 cell line isolated from hormone-dependent mammary carcinoma - MCCLX). Previous evidence suggested that an unknown phospholipid of pituitary origin could stimulate the proliferation and growth rate of these tumor cells. To test this hypothesis, the authors exposed the tumor cells at different concentrations of compound (0.2 to 50 nmol/mL). The tests indicated that PHOS-S induces tumor growth in a dose-dependent manner (Kano-Sueoka *et al.*, 1979).

## DISCUSSION

A subset of the analyzed studies converged to a hypothesis that PHOS-S has anticarcinogenic potential. Assessments of the influence of this compound on

the activity of mononuclear phagocyte system cells (macrophages), which are important for neoplastic development (Laoui *et al.*, 2014), indicated that PHOS-S has inhibitory activity in these cells.

Moreover, in some of the analyzed studies, cytotoxic and inhibitory effects of PHOS-S were observed in tumor cells. These results theoretically confirm evidence that phospholipids have anticarcinogenic activities (Koufaki *et al.*, 1996; Hochhuth *et al.*, 1990). Finally, the study by Ferreira and colleagues (2013c) of the antileukemic properties of PHOS-S also corroborates findings by Dhakshinamoorthy *et al.* (2015) of a possible role of PHOS-S in the inhibition of leukemic cells.

However, most of the studies of the possible anticarcinogenic effects of PHOS-S that were analyzed in this review were from the same research group. In addition, the *in vivo* and *in vitro* results are controversial when compared with other sources of information (Ferreira *et al.*, 2013c; Brasil, 2013; Brasil, 2016a; Brasil, 2016b).

Reports commissioned by the Brazilian's Ministry of Science, Technology and Innovation showed contrary implications of the anticarcinogenic effects of this substance. The experiments carried out in these reports (even using extremely high concentrations of the compound) did not support PHOS-S as an inhibitor of cancer (Brasil, 2013, 2016a,b). Finally, conflicting results were obtained by Kano-Sueoka and colleagues in a study published in 1979 and conducted in the USA (the only study outside Brazil).

It is also important to analyze the limitations of the studies published to date. As discussed at the beginning of this article, the analysis of the properties of PHOS-S is still based exclusively on preclinical animal or cellular models. Clinical studies have started recently, and they are still in a reduced scale (Pivetta, 2016). The use of a drug based exclusively on preclinical studies should be avoided. This was not the case with PHO-S, which began to be used in humans even without further clinical studies (Escobar, 2016).

## CONCLUSION

A portion of the analyzed studies suggest possible anticarcinogenic potential of PHOS-S. However, the limitations of the published studies must be considered. In the literature, we identified only preclinical studies (in cells or in animals). No clinical study in humans has been published in the literature until the present moment.

The lack of more complex studies in humans does not allow a more in-depth and qualitative analysis of the actual toxicological implications of the compound.

Even though PHOS-S did indeed have anticarcinogenic properties, there was insufficient evidence that the drug would not negatively affect its users. Thus, it's certain to assert that an analysis of the actual implication of using this compound as an anticancer drug will still take time.

Therefore, it is necessary to evaluate the real implications of PHOS-S as a possible drug for use in cancer treatments. Further studies are needed on a broader scale to assert the legitimate anticarcinogenic capability of this compound.

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## REFERENCES

- Anastácio LB, Oliveira DA, Delmaschio CR, Antunes LMG, Chequer FMD. Corantes alimentícios amarantho, eritrosina B e tartrazina, e seus possíveis efeitos maléficis à saúde humana. *J Appl Pharm Sci.* 2016;2(3):16-30.
- Arruda MSP, Correa MA, Venturini J, Félix MC, De Rosis AMB, Galhiane MS, et al. The effect of phosphoethanolamine intake on mortality and macrophage activity in mice with solid Ehrlich tumors. *Brazilian Arch Biol Technol.* 2011;54(6):1203-10.
- Bakovic M, Fullerton MD, Michel V. Metabolic and molecular aspects of ethanolamine phospholipid biosynthesis: the role of CTP: phosphoethanolamine cytidyltransferase (Pcyt2). *Biochem Cell Biol.* 2007;85(3):283-300.
- Boyle P, Levin B. World cancer report, 2008. *Int Agency Res Cancer;* 2008. [cited 2016 Jul 15]. Available from: [http://www.iarc.fr/en/publications/pdfs-online/wcr/2008/wcr\\_2008.pdf](http://www.iarc.fr/en/publications/pdfs-online/wcr/2008/wcr_2008.pdf)
- Brasil. Ministério da Ciência, Tecnologia, Inovações e Comunicações. Resumo Executivo: avaliação da atividade citotóxica e antiproliferativa da fosfoetanolamina, monoetanolamina e fosfobisetanolamina em células humanas de carcinoma de pâncreas e melanoma. Florianópolis; 2013.
- Brasil. Universidade Federal do Ceará. UFCE. Faculdade de Medicina. Núcleo de pesquisa e desenvolvimento de medicamentos. Laboratório de oncologia Experimental. Laudo técnico do estudo da fosfoetanolamina sintética: Avaliação da possível atividade anticâncer da fosfoetanolamina sintética (FS) no sarcoma 180. Fortaleza; 2016a.

Brasil. Universidade Federal do Ceará. UFCE. Faculdade de Medicina. Núcleo de pesquisa e desenvolvimento de medicamentos. Laboratório de oncologia Experimental. Laudo técnico do estudo da fosfoetanolamina sintética: Avaliação da possível atividade anticâncer da fosfoetanolamina sintética (FS) no carcinossarcoma 256 de Walker. Fortaleza; 2016b.

Dhakshinamoorthy S, Dinh N-T, Skolnick J, Styczynski MP. Metabolomics identifies the intersection of phosphoethanolamine with menaquinone-triggered apoptosis in an in vitro model of leukemia. *Mol BioSyst.* 2015;11(9):2406-16.

Doll R, Peto R. The causes of cancer: Quantitative estimates of avoidable risks of cancer in the united states today. *J Natl Cancer Inst.* 1981;66(6):1192-308.

Escobar H. Brazil president signs law legalizing renegade cancer pill. *Science Magazine*; 2016. [cited 2016 Dec 02]. Available from: <http://www.sciencemag.org/news/2016/04/brazil-president-signs-law-legalizing-renegade-cancer-pill>.

Elbayoumi T, Shen R, Kim J, Yao M. Development and evaluation of vitamin E D- $\alpha$ -tocopheryl polyethylene glycol 1000 succinate-mixed polymeric phospholipid micelles of berberine as an anticancer nanopharmaceutical. *Int J Nanomedicine.* 2016;11:1687-700.

Ferreira AK. Alquil fosfatado sintético precursor dos fosfolipídios de membrana celular com potencial efeito antitumoral e apoptótico em modelos de tumores experimentais [Tese doutorado]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2013.

Ferreira AK, Meneguelo R, Neto SC, Chierice GO, Maria DA. Synthetic phosphoethanolamine induces apoptosis through caspase-3 pathway by decreasing expression of Bax/Bad protein and changes cell cycle in melanoma. *J Cancer Sci Ther.* 2011;3(3):53-9.

Ferreira AK, Meneguelo R, Pereira A, Mendonça Filho OR, Chierice GO, Maria DA. Anticancer effects of synthetic phosphoethanolamine on Ehrlich ascites tumor: an experimental study. *Anticancer Res.* 2012a;32(1):95-104.

Ferreira AK, Meneguelo R, Marques FLN, Radin A, Filho OMR, Neto SC, et al. Synthetic phosphoethanolamine a precursor of membrane phospholipids reduce tumor growth in mice bearing melanoma B16-F10 and in vitro induce apoptosis and arrest in G2/M phase. *Biomed Pharmacother.* 2012b;66(7):541-8.

Ferreira AK, Meneguelo R, Pereira A, Filho OMR, Chierice GO, Maria DA. Synthetic phosphoethanolamine induces cell cycle arrest and apoptosis in human breast cancer MCF-7 cells through the mitochondrial pathway. *Biomed Pharmacother.* 2013a;67(6):481-7.

Ferreira AK, Freitas VM, Levy D, Ruiz JLM, Bydlowski SP, Rici REG, et al. Anti-angiogenic and anti-metastatic activity of synthetic phosphoethanolamine. *PLoS One.* 2013b;8(3):e57937.

Ferreira AK, Santana-Lemos BAA, Rego EM, Filho OMR, Chierice GO, Maria DA. Synthetic phosphoethanolamine has in vitro and in vivo anti-leukemia effects. *Br J Cancer.* 2013c;109(11):2819-28.

Gullotti E, Yeo Y. Targeted Drug Delivery. *Mol Pharm.* 2010;6(4):1041-51.

Hochhuth C, Berkovic D, Eibl H, Unger C, Doenecke D. Effects of antineoplastic phospholipids on parameters of cell differentiation in U937 cells. *J Cancer Res Clin Oncol.* 1990;116(5):459-66.

Kano-Sueoka T, Cohen DM, Yamaizumi Z, Nishimura S, Mori M, Fujiki H. Phosphoethanolamine as a growth factor of a mammary carcinoma cell line of rat. *Proc Natl Acad Sci.* 1979;76(11):5741-4.

Koufaki M, Polychroniou V, Calogeropoulou T, Tsoinias A, Drees M, Fiebig HH, et al. Alkyl and Alkoxyethyl Antineoplastic Phospholipids. *J Med Chem.* 1996;39(13):2609-14.

Laoui D, van Overmeire E, de Baetselier P, van Ginderachter JA, Raes G. Functional relationship between tumor-associated macrophages and macrophage colony-stimulating factor as contributors to cancer progression. *Front Immunol.* 2014;5:1-15.

Macquart-Moulin G, Viens P, Bouscary ML, Genre D, Resbeut M, Gravis G, et al. Discordance between physicians' estimations and breast cancer patients' self-assessment of side-effects of chemotherapy: an issue for quality of care. *Br J Cancer* 1997;76(12):1640-5.

Meneguelo, R. Efeitos antiproliferativos e apoptóticos da fosfoetanolamina sintética no melanoma B16F10 [Dissertação mestrado]. Escola de Engenharia de São Carlos, Faculdade de Medicina de Ribeirão Preto, Instituto de Química de São Carlos, Universidade de São Paulo; 2007.



- Moher D, Liberati A, Tetzlaff J, Altman DG, Prisma Group. Preferred reporting items for systematic reviews and meta-analyses : the PRISMA statement. *Plos Med.* 2009;339(august):332-6.
- Outhouse EL. Amino-ethyl phosphoric ester from tumours. *Biochem J.* 1936;30(2):197-201.
- Pivetta, M. A prova final da fosfoetanolamina. *Revista FAPESP.* 2016;243:16-23.
- Pondé N, Ades F, Azambuja E. Threat posed by unproven drugs in medical oncology. *ESMO Open.* 2016;1:4-6.
- Sarraf JS, Câmara TF, Puty TC, Carvalho LEW. Uso inadvertido da fosfoetanolamina sintética no Brasil: Por que se preocupar? *Rev Bras Cancerol.* 2016;62(1):47-50.
- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin.* 2016;66(1):7-30.
- Sikora K, Advani S, Korolchouk V, Magrath I, Levy L, Pinedo H, et al. Essential drugs for cancer therapy: a World Health Organization consultation. *Ann Oncol.* 1999;10(4):385-90.
- Veronez LC. Atividade da Fosfoetanolamina Sintética em Melanoma Murino Experimental [Dissertação de mestrado]. Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo; 2012.
- Vidal M, Carvalho C, Bispo R. Use of complementary and alternative medicine in a sample of women with breast cancer. *SAGE Open.* 2013;3(3):2013-6.
- Weinberg RA. The rational treatment of cancer. In: *The biology of cancer.* New York: Garland Science; 2014.
- World Health Organization. WHO. Cancer; 2017. [cited 2017 Apr 6]. Available from: <http://www.who.int/cancer/en/>.
- World Health Organization. WHO. Policies and managerial guidelines for national cancer control programs. *Rev Panam Salud Publica* 2002;12(5):366–70. DOI: 10.1590/S1020-49892002001100015.
- Yu Y, Kanwar SS, Patel BB, Nautiyal J, Sarkar FH, Majumdar AP. Elimination of colon cancer stem-like cells by the combination of curcumin and FOLFOX. *Transl Oncol.* 2009;2(4):321-8.

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