SCIENTIFIC ARTICLE

Effect of ultra-diluted nux vomica and cyclophosphamide solutions on the genotoxicity of allopathic cyclophosphamide

Efeito das soluções ultradiluídas de nux vomica e ciclofosfamida na genotoxicidade da ciclofosfomida alopática

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Abstract

Homeopathic medicines comprise the use of pharmacotechnical processes that promote successive dilutions, followed by grinding or agitation of compounds. The purpose of this study was to assess the genotoxic potential of ultra-diluted cyclophosphamide (CF) and nux vomica associated with or without allopathic CF in Swiss Webster mice, using the micronucleus test. The compounds were prepared according to the Brazilian homeopathic pharmacopoeia. Swiss Webster mice were randomly divided into eight groups (n=6) according to the compounds to be tested, and received by gavage (vehicle or CF) or by oral mucosa contact (ultra-diluted CF and nux vomica, and dynamized solutions) once a day for 7 days. After this, one of the groups treated with the dynamized compound was challenged by the administration of the allopathic CF. The dynamized ultra-diluted nux vomica and CF compounds showed lower white cell counts in mice. However, no compound was able to mitigate the genotoxic effects of CF in micronucleus assay. The dynamized compounds did not cause damage to the spleen and thymus, and when used intraperitoneally, they were able to mitigate the effect of CF on thymic cortical reduction in mice. Further studies with nux vomica and dynamized CF should be performed to better delineate their possible therapeutic potential in reducing adverse effects on chemotherapy.

Keywords: homeopathy, cyclophosphamide, nux vomica, micronucleus, genotoxicity.

Resumo

Os medicamentos homeopáticos compreendem a utilização de processos farmacotécnicos que promovem diluições sucessivas seguidas de trituração ou agitação dos compostos. O objetivo deste estudo é avaliar o potencial genotóxico da ciclofosfamida UD e nux vomica UD associada ou não à ciclofosfamida (C) alopática em camundongos Swiss Webster, por meio do teste de micronúcleo. Os compostos foram preparados de acordo com a Farmacopéia Homeopática Brasileira. Camundongos Swiss Webster foram divididos randomicamente em oito grupos (n = 6) de acordo com os compostos a serem testados, e receberam os compostos através de gavagem (Veículo ou CF) ou administração por contato da mucosa oral (Ciclofosfamida e nux Vomica soluções ultradiluídas e dinamizadas), uma vez por dia, durante 7 dias. Em seguida, um dos grupos tratados com o composto dinamizado foi desafiado pela administração da ciclofosfamida alopática. Os compostos nux vomica e ciclofosfamida ultradiluídos dinamizados apresentaram menores valores na contagem de leucócitos em camundongos. No entanto, nenhum composto foi capaz de mitigar os efeitos genotóxicos da ciclofosfamida no ensaio de micronúcleo. Os compostos dinamizados não causaram danos ao baço e ao timo e, quando usados por via intraperitoneal, demonstraram ser capazes de mitigar o efeito da ciclofosfamida na redução da cortical do timo em camundongos. Mais estudos com nux vomica e ciclofosfamida dinamizada devem ser realizados para melhor delinear seu potencial terapêutico na redução de efeitos adversos em quimioterapia.

Palavras-chave: homeopatia, ciclofosfamida, nux vomica, micronúcleo, genotoxicidade.

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Introduction

Homeopathy is a medical system officially recognized in many countries (Dantas, 2004), and its national regulatory framework and location within the health care system differ from country to country. This therapy is one of the complementary medicines recognized by the World Health Organization (WHO) and is based on these four basic principles: (1) Similar Law (Similia Similibus Curantur), known by the expression "like is cured by like," in which a disease may be cured by something that can cause similar symptoms; (2) medicinal experiment in Healthy Men, an experimental methodology aimed at identifying toxicological properties of the substance to be further applied by the principle of similarity, and which will be part of the homeopathic medical matter; (3) infinitesimal doses, which is a pharmacological technique that employs successive dilutions and dynamizations in order to eliminate toxicity and preserve the substance's medicinal properties; and (4) single medicine, which is the substance of homeopathic medical matter that presents an experimental symptomatic totality similar to the symptomatic totality presented by a patient (Note: Homeopathic Medical Matter consists of substances or elements from the Mineral, Plant, and Animal Kingdoms - Farmacopeia Homeopática Brasileira (Agência Nacional de Vigilância Sanitária, 2011).

The classical experimental methodology of homeopathic medicine is advocated based on the second principle. For this purpose, the identification of diluted and dynamized solutions that induce symptoms in a healthy organism and whose symptomatic set similar to the natural disease, is the criteria for the therapeutic indication of its use. This methodology presents operational difficulties, requiring an adjustment and adequation to the current scientific methodology (Dantas, 2004; Pinto, 1995; Pitari, 2007; Pinto, 2008a; Pinto, 2008b; Pinto, 2009; Posadzki & Ernst, 2013; Rosas, 2006). However, international agencies and government institutions accept the micronucleus assay as part of the recommended test battery to establish the evaluation and registration of new chemicals and pharmaceutical products that enter the world market annually (Choy, 2001). This assay was developed to identify chemical substances that caused damage to cellular genetic material, into which the test substance would be administered to the animal under sub-acute treatment, and its effect was measured by counting the micronucleated cell frequency in bone marrow smears (Borges et al., 2019; Schmid, 1976; MacGregor et al., 1987; Norppa & Falck, 2003; Organisation for Economic Cooperation and Development, 1997). Currently, this test is widely used to evaluate the genotoxic effects of substances in vivo (Borges et al., 2019; Hussain, et al., 2020; Khayyat et al., 2017) or possible protective effects of certain substances on genotoxic induction of mutagenic drugs (Hussain et al., 2018).

Some agents mainly used in antineoplastic and immunosuppressive therapies, like cyclophosphamide (CF) have a clear genotoxic effect. CF acts by alkylating cellular constituents, leading to DNA cross-linking and disruption of transition and translation, thereby replacing a hydrogen atom with an alkyl radical, binding to the DNA to preclude the separation of two filaments of the molecule in the double helix, which is an indispensable process for replication. Thus, it affects the cells in all cell cycle phases in a nonspecific manner. Therefore, chemotherapy is classified as a cycle-specific compound that acts only on proliferating cells (Instituto Nacional do Câncer, 2015), causing adverse effects on the hematopoietic system and gastrointestinal tract, which may produce secondary tumors due to its genotoxicity (Bruni et al., 2019; Schuurman et al., 2005). The most common side effects are related to the gastrointestinal tract, in addition to spinal cord depletion, infertility, and hemorrhagic cystitis (Bruni et al., 2019; Garas et al., 1995; Green et al., 1989; Knysak et al., 1994; Murti & Horsman, 1979). As the DNA molecule is the major target of genotoxic agents, these changes can result in cell dysfunctions, such as genetic instability and mutagenesis (Ferreira, 2008). Undoubtedly, the effects of chemotherapeutic agents depend on the exposure time and the compound's plasma concentration, which is their toxicity variable for the different tissues and drugs used (Almeida et al., 2005). The search for new therapeutic approaches to inhibit or minimize these effects is of paramount importance and, within Homeopathic Medicine, Strychnos *Nux Vomica*, the walnut vomica stands out (Guo et al., 2018). In addition, pursuant to the Brazilian College Board Resolution (ANVISA, 2007), it is considered to be a dynamized medicinal product: preparations made from substances that are subjected to successive crushing or dilution followed by succussion, or another form of rhythmic agitation, for preventive or curative purposes, to be administered according to homeopathic, homotoxicological, and anthroposophic therapy. Thus, the dynamized form of CF may be subjected to dilution followed by succussion, to be administered according to homeopathic therapy.

Considering the homeopathic treatment, the compound nux vomica has been widely used today to assist in the treatment of cancer patients, in order to reduce adverse effects in patients receiving anti-neoplastic chemotherapy, including CF. It is used as the homeopathic medicine of choice mainly to reduce nausea (Kassab et al., 2009). Based on these studies, there is evidence that homeopathic medicine can help manage the side effects of cancer treatment.

Despite the fact that there is some criticism of these products being contrary to all requirements of modern pharmacology, patients have reported positive effects from homeopathic therapy (World Health Organization, 2009) and the use of homeopathic medicines continues to spread. Therefore, studies to understand the effect of the use of these remedies and their impact on human and animal health as well as the safety and quality of homeopathic medicines have become a major concern.

The aim of this study was to experimentally evaluate the effect and possible toxicity of nux vomica homeopathic compounds and dynamized CF in Swiss Webster mice, as well as their effect on the genotoxic potential of allopathic CF. For this, body and hematological parameters, frequency of micronuclei, and occurrence of alteration in the incidence of polychromatic erythrocytes (PCE) in the bone marrow as well as the macro and microscopic findings in the spleen and thymus of the treated animals were observed.

Material and methods

Ultra-diluted compounds

Ultra-diluted CF and nux vomica were prepared according to the preparation and control methods described in the Brazilian Homeopathic Pharmacopoeia, 3° Edição (Agência Nacional de Vigilância Sanitária, 2011).

Animal Treatment: Forty female Swiss Webster mice weighing 18-20 g from the Instituto de Ciência e Tecnologia em Biomodelos (ICTB) da Fundação Oswado Cruz were used for the study. The animals were randomly divided into eight groups (n=06) according to the compounds to be tested, weighed, and kept for approximately 1 week prior to the commencement of the experiments. At the beginning of the treatment, the animals were weighed again and received different compounds by gavage or oral mucosa contact (in the case of ultra-diluted solutions) once a day for 7 days. At the end of the experiment, the animals were euthanized, and the organs were sampled.

The animals were distributed into eight groups: S/TTO (without any treatment); VE (animals received only the vehicle); CF (animals received only the CF); NVd (animals received only Dynamized nux vomica); CFd (animals received only Dynamized CF); NVd+CF (animals received Dynamized nux vomica for 7 days and CF at the end); CFd/VO+CF (animals received Dynamized CF by oral route for 7 days and CF at the end); CFd/IP+CF (animals received dynamized CF by intraperitoneal route for 7 days and CF at the end).

Complete blood count analysis (CBC) and histopathology

Following euthanasia, blood was collected for hematological analysis through intracardiac puncture, and thereafter, necroscopic examination of the animals was performed. The following organs were removed and weighed: liver, spleen, right kidney, and thymus. After weighing, the respective hepatic, splenic, renal, and thymic indices were calculated as (absolute organ weight × 100%)/ body weight of mice. The organs were fixed in 10% formaldehyde solution in PBS buffer and further processed by standard paraffin embedding techniques and hematoxylin-eosin staining.

Micronucleus assay

The micronucleus test was performed on bone marrow samples of the animals. The right femur was collected from each animal with subsequent removal of the epiphyses, followed by washing of the medullary canal with 1.0mL of fetal bovine serum (FBS) at room temperature. The collected material was centrifuged (1200rpm/10 min), the supernatants were discarded, and the pellet was resuspended with FBS, and Giemsa-stained smears were prepared for observation using optical microscopy. The evaluation of clastogenic and/or aneugenic potential was performed from the analysis of two thousand polychromatic erythrocytes (PCE) per animal, and the frequency of

micronucleated PCE (MNPCE) in 200 erythrocytes was also recorded. Analyses were performed under a light microscope at 1000X magnification.

Statistical analysis

Means values and standard errors were used to describe the numerical variables. The comparison between the groups' means was performed by analysis of variance (ANOVA), followed by Dunnett's post-test with the CF group or the S/TTO group as the control group, depending on the evaluated variable. The significance value was assumed to be $P<0.05$.

Results

All animals had an increase in body mass during the experiment ($P<0.0001$), and there was no difference in body mass at the end of the experiment (Figure 1). Regarding the hematological analysis data, it was observed that only the S/TTO and VE groups presented values significantly higher than the CF group with respect to the global white blood cells count (Table 1) ($P=0.000$ and $P=0.023$ respectively). For all the other parameters, there was no difference in relation to the S/TTO group.

Relation between organ weights and body weight

As for the liver, only the group that received dynamized cyclophosphamide had a different mean than the group that received allopathic CF in relation to the liver index ($P=0.006$). In the spleen, the groups that had splenic indices mean different from the CF group were: dynamized CF treated group ($P=0.002$), dynamized nux vomica treated group ($P=0.002$), vehicle treated group ($P=0.004$), and vehicle treated group ($P=0.009$). Regarding the thymic index, there was a difference between the groups treated with CF ($P=0.005$) and the group without treatment ($P=0.018$). There was no difference in kidney weight (Figure 2).

Histopathology

In the spleen, the degree of splenic alteration was assessed by observing the reduction of extramedullary hematopoiesis with consequent reduction of red pulp, and was classified as 0, 1, 2, or 3 according to its intensity. This graduation scale was also used to evaluate the thymus in relation to the cortical reduction of this organ. The results are shown in Figures 3, 4, 5, and 6.

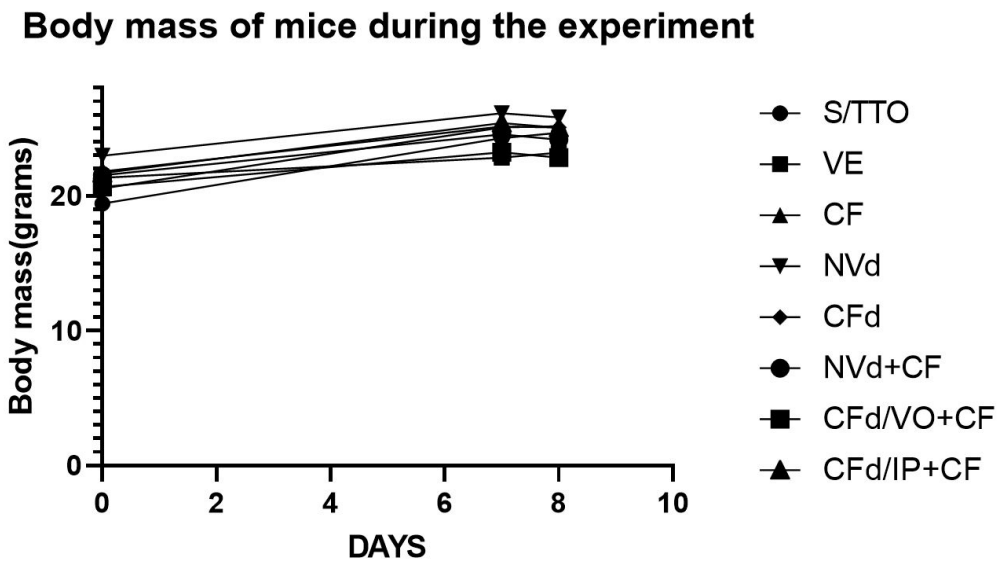

Figure 1. Impact of dynamized ultra-diluted nux vomica and Cyclophosphamide compounds in Body mass (grams - Mean and Standard error).

Caption: S/TTO (without any treatment); VE (animals received only the vehicle); CF (animals received only the CF); NVd (animals received only Dynamized nux vomica); CFd (animals received only Dynamized CF); NVd+CF (animals received Dynamized nux vomica for 7 days and CF at the end); CFd/VO+CF (animals received Dynamized CF orally for 7 days and CF at the end); CFd/IP+CF (animals received Dynamized CF by intraperitoneal route for 7 days and CF at the end).

Table 1. Effects of dynamized ultra-diluted Nux Vomica and Cyclophosphamide compounds in Complete blood count analysis (CBC) (Mean and standard error).

Groups	RBC count (million cells/ mm³)	Hb (g/dL)	Htc (%)	MCV (fm³)	MCH (pg)	MCHC (g/dL)	WBC count (x10³/ mm³)	Platelets (x10³/mm³)
S/TTO	9.0 ± 0.19	14.4 ± 0.08	52.6 ± 0.77	58.5 ± 0.38	16.0 ± 0.27	27.4 ± 0.31	16.9 ± 1.67 **	1,371.8 ± 39.36
VE	9.0 ± 0.62	15.2 ± 0.21	55.4 ± 0.72	57.7 ± 0.87	15.8 ± 0.23	27.4 ± 0.12	14.5 ± 1.37 *	1,390.6 ± 31.69
CF	9.1 ± 0.16	14.2 ± 0.26	50.9 ± 1.08	56.2 ± 0.37	15.7 ± 0.06	27.9 ± 0.09	9.6 ± 0.44	1,396.4 ± 27.44
NVd	9.4 ± 0.29	14.6 ± 0.33	54.1 ± 1.3	57.9 ± 0.52	15.6 ± 0.17	27.0 ± 0.24	12.5 ± 0.84	1,418.4 ± 129.86
CFd	9.4 ± 0.22	14.7 ± 0.36	53.3 ± 1.44	55.7 ± 1.24	15.7 ± 0.29	27.5 ± 0.37	14.2 ± 1.52	1,386.3 ± 141.11
NVd+CF	9.3 ± 0.13	14.3 ± 0.05	51.7 ± 0.54	55.4 ± 0.85	15.3 ± 0.21	27.6 ± 0.23	8.2 ± 1.19	1,269.6 ± 74.43
CFd/VO+CF	9.0 ± 0.08	14.0 ± 0.25	50.2 ± 0.89	55.9 ± 0.88	15.6 ± 0.26	28.0 ± 0.12	6.5 ± 0.75	1,231.6 ± 103.54
CFd/IP+CF	8.9 ± 0.19	14.0 ± 0.33	50.4 ± 1.43	55.6 ± 1.02	15.9 ± 0.32	28.0 ± 0.25	5.7 ± 0.65	1,211.8 ± 86.15

Caption: S/TTO (without any treatment); VE (animals received only the vehicle); CF (animals received only the CF); NVd (animals received only Dynamized nux vomica); CFd (animals received only Dynamized CF); NVd+CF (animals received Dynamized nux vomica for 7 days and CF at the end); CFd/VO+CF (animals received Dynamized CF orally for 7 days and CF at the end); CFd/IP+CF (animals received Dynamized CF by intraperitoneal route for 7 days and CF at the end); *Statistically significant difference (P<0.05) compared to the CF group; **Statistically significant difference (P<0.01) compared to the CF group; RBC, red blood cell; Hb, Hermoglobin; Htc, Hematocrit; MVC, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; WBC, white blood cell.

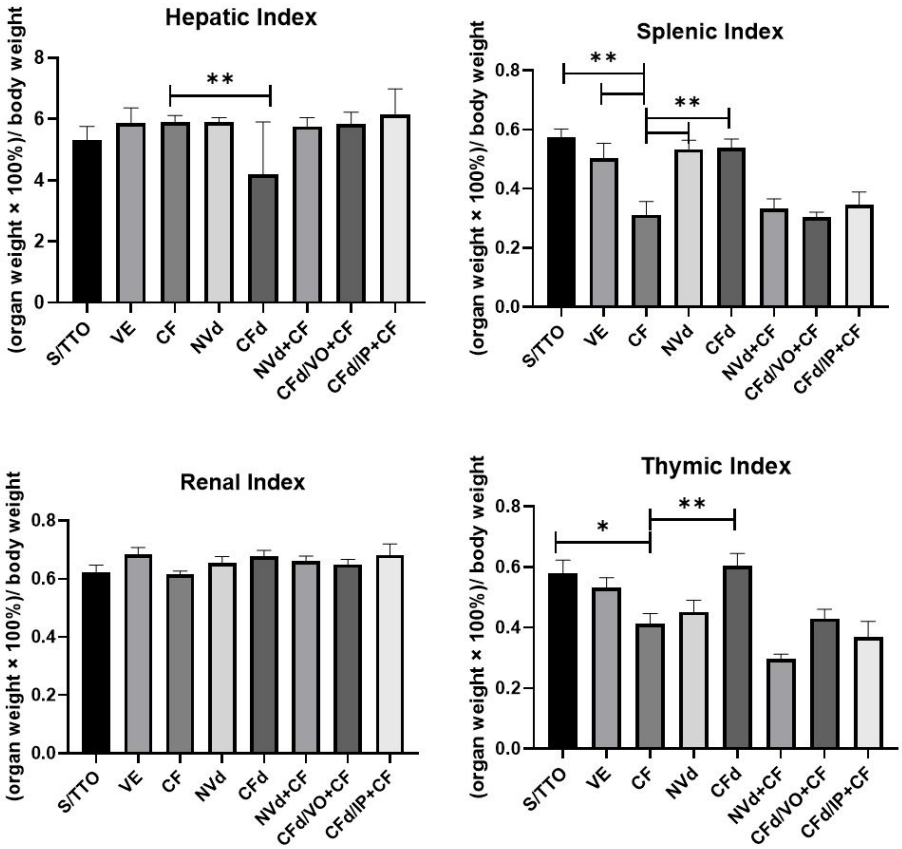

Figure 2. Dynamized ultra-diluted nux vomica and Cyclophosphamide compounds effect in Hepatic, splenic, thymic, and renal indices.

Caption: * Statistically significant difference (P<0,05) compared to the CF group. ** Statistically significant difference (P<0,01) compared to the CF group. S/TTO (without any treatment); VE (animals received only the vehicle); CF (animals received only the CF); NVd (animals received only Dynamized nux vomica); CFd (animals received only Dynamized CF); NVd+CF (animals received Dynamized nux vomica for 7 days and CF at the end); CFd/VO+CF (animals received Dynamized CF orally for 7 days and CF at the end); CFd/IP+CF (animals received Dynamized CF by intraperitoneal route for 7 days and CF at the end).

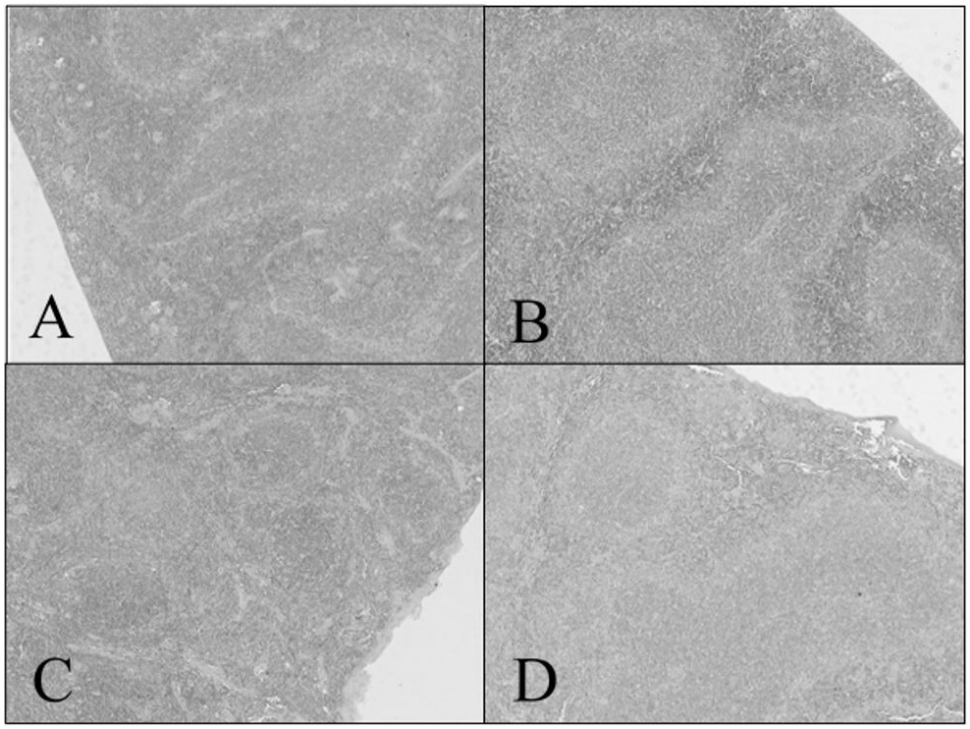

Figure 3. Mice. Spleen. Representative optical microscopy images of the different degrees of splenic hematopoietic changes. (A) Grade 0 (s/TTO group); (B) Grade 1 (NVd group); (C) Grade 2 (CFd/IP+CF); (D) Grade 3 (CF group). (H.E. 100x).

Captions: S/TTO (without any treatment); NVd (animals received only dynamized nux vomica); CFd/IP+CF (animals received dynamized CF by intraperitoneal route for 7 days and CF at the end).

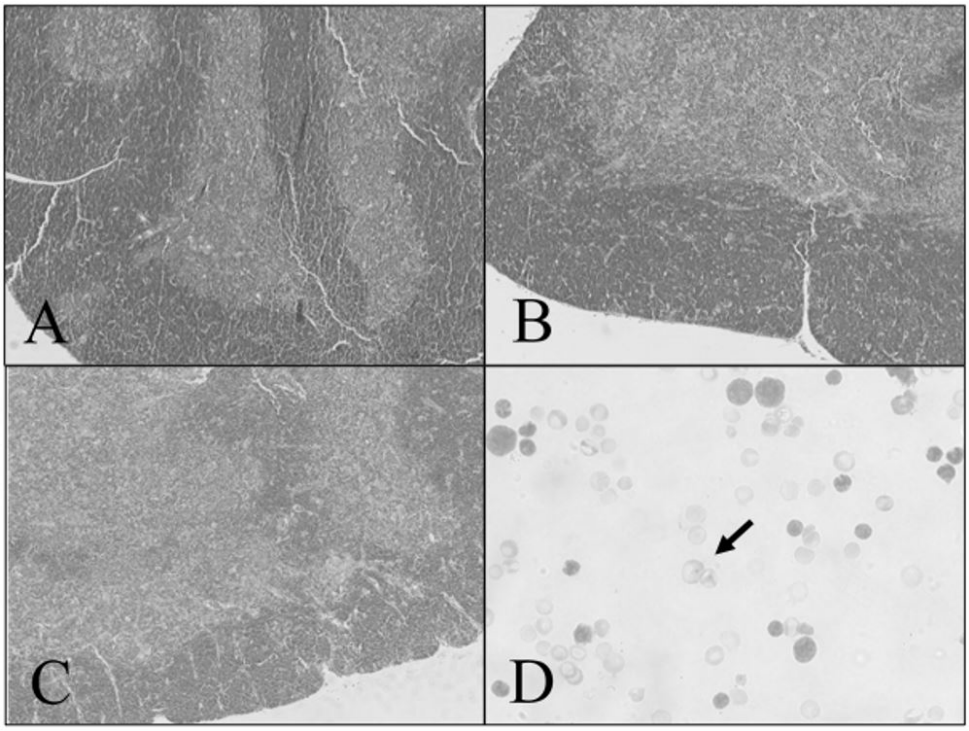

Figure 4. Mice. Thymus. Representative optical microscopy images of the different degrees of thymic cortical reduction and micronucleus. (A) Grade 0 (S/TTO group); (B) Grade 1 (CFd/IP+CF group); (C) Grade 2 (CF group). (H.E. 100x); (D) Polychromatic erythrocytes with micronucleus (arrow) (Giemsa; 1000x).

Captions: S/TTO (without any treatment); CFd/IP+CF (animals received dynamized CF by intraperitoneal route for 7 days and CF at the end).

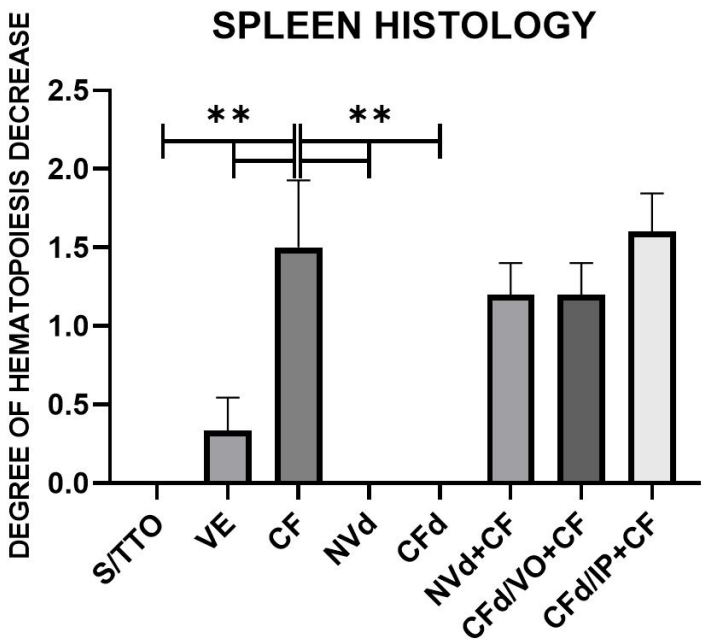

Figure 5. Dynamized ultra-diluted nux vomica and Cyclophosphamide compounds effects on average grade of splenic hematopoiesis involvement.

Caption: S/TTO (without any treatment); VE (animals received only the vehicle); CF (animals received only the CF); NVd (animals received only Dynamized nux vomica); CFd (animals received only Dynamized CF); NVd+CF (animals received Dynamized nux vomica for 7 days and CF at the end); CFd/VO+CF (animals received Dynamized CF orally for 7 days and CF at the end); CFd/IP+CF (animals received Dynamized CF by intraperitoneal route for 7 days and CF at the end).** Statistically significant difference (P<0.01) compared to the CF group.

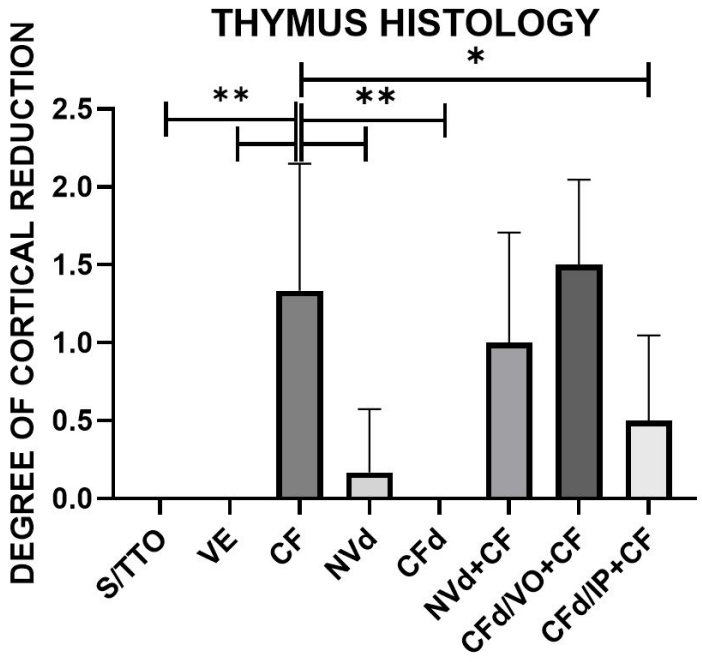

Figure 6. Dynamized ultra-diluted nux vomica and Cyclophosphamide compounds effects on average grade of thymic cortical reduction.

Caption: S/TTO (without any treatment); VE (animals received only the vehicle); CF (animals received only the CF); NVd (animals received only Dynamized nux vomica); CFd (animals received only Dynamized CF); NVd+CF (animals received Dynamized nux vomica for 7 days and CF at the end); CFd/VO+CF (animals received Dynamized CF orally for 7 days and CF at the end); CFd/IP+CF (animals received Dynamized CF by intraperitoneal route for 7 days and CF at the end). * Statistically significant difference (P<0,05) compared to the CF group. ** Statistically significant difference (P<0,01) compared to the CF group.

The untreated groups and vehicle-treated, nux vomica - treated and dynamically CF-treated groups had lower means than the control group treated with CF (P<0.01) in the spleen.

Regarding the thymus, the untreated groups and vehicle-treated, nux vomica-treated and dynamically CF-treated groups as well as the group treated with intraperitoneally dynamized CF and challenged had lower means than the control group treated with CF (P<0.01 for the non-challenged and P<0.05 for the treated with intraperitoneally dynamized CF and cyclophosphamide.

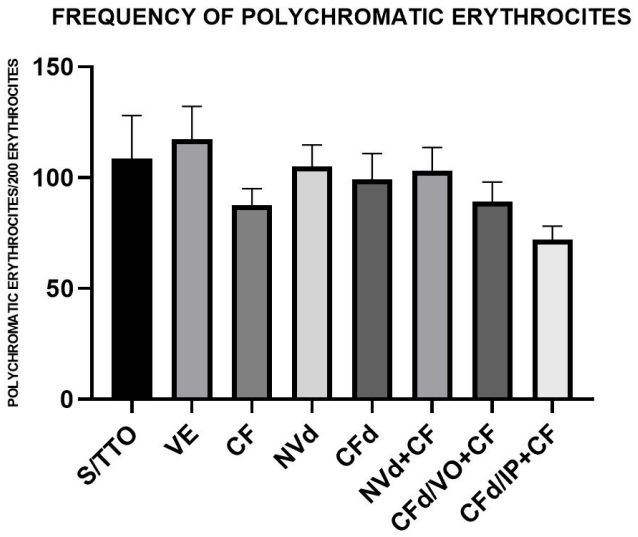

Figure 7. Frequency of polychromatic erythrocytes (poly/200) after dynamized ultra-diluted Nux Vomica and Cyclophosphamide treatment.

Caption: S/TTO (without any treatment); VE (animals received only the vehicle); CF (animals received only the CF); NVd (animals received only Dynamized nux vomica); CFd (animals received only Dynamized CF); NVd+CF (animals received Dynamized nux vomica for 7 days and CF at the end); CFd/VO+CF (animals received Dynamized CF orally for 7 days and CF at the end); CFd/IP+CF (animals received Dynamized CF by intraperitoneal route for 7 days and CF at the end).

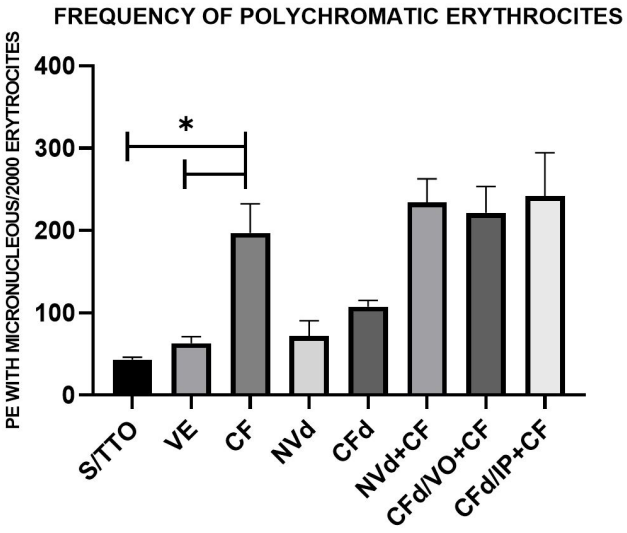

Figure 8. Frequency of micronucleus in polychromatic erythrocytes.

Caption: S/TTO (without any treatment); VE (animals received only the vehicle); CF (animals received only the CF); NVd (animals received only Dynamized nux vomica); CFd (animals received only Dynamized CF); NVd+CF (animals received Dynamized nux vomica for 7 days and CF at the end); CFd/VO+CF (animals received Dynamized CF orally for 7 days and CF at the end); CFd/IP+CF (animals received Dynamized CF by intraperitoneal route for 7 days and CF at the end). * Statistically significant difference (P<0.05) compared to the CF group. # P=0.05 compared to the CF group.

Micronucleus Test

Regarding the micronucleus assay, the frequency of polychromatic erythrocytes did not vary between the groups studied (Figure 7) and the frequency of micronuclei (Figure 4D and 8); only the untreated groups and the group that received vehicle had significantly lower means than the CF-treated group (P<0.05). The mean of the group treated with dynamized nux vomica showed a tendency towards this value (P=0.05).

Discussion

Animal models are still fundamental for research in homeopathy, in the evaluation of toxicity (Gupta et al., 2016; Khuda-Bukhsh, 2009), and in the carcinogenic potential of compounds (Khuda-Bukhsh, 2009). Thus, in the present study, mice (*Mus musculus*) were used as an animal model to evaluate the effect of ultradiluted nux vomica e Cyclophosphamide on the allopathic CF genotoxicity.

Several studies, like the present study, aimed to observe the effects or the reduction of the effects of allopathic drugs or other drugs with the administration of homeopathic medicines. Khuda-Bukhsh (2009) observed a reduction in genotoxic and DNA damage in bone marrow cells of mice that were treated with cutaneous papilloma with potentiated homeopathic drugs. Other studies have shown that ultra-low doses of certain homeopathic medicines have both antigenotoxic (Biswas & Khuda-Bukhsh, 2002) and induce genotoxicity in animals (Preethi et al., 2008). Nascimento and collaborators (2016) demonstrated in experimental conditions (*in vitro*) that CANOVA has antigenotoxic and anti-cytotoxic effects in human lymphocytes, when exposed to a carcinogenic alkylating agent. However, it was also observed that CANOVA reduced the size of the tumor and/or caused regression of the cancer in many cases, in addition to the low cost (Seligmann et al., 2003) and other studies that were successful with its use (Buchi & Del Vecchio, 2002; Piraíno, 2003). Biswas et al. (2005) reported the relative efficacy of Carcinossin 200 (Car 200), administered alone and in combination with Chelidonium 200 (Ch 200), in improving the picture of p-DAB-induced hepatocarcinogenesis in mice.

In addition to the various aspects related to therapeutic use, Banerjee et al. (2007) raised a problem that affected the entire planet as a justification for the development of their study: the contamination of groundwater by arsenic without an effective drug to combat chronic poisoning. These authors demonstrated the importance of the anti-mutagenic role played by a potentiated homeopathic compound, "Arsenicum Album-200" in a study carried out in mice chronically poisoned by arsenic. As in the current study, toxicity tests were developed, such as the presence or absence of micronuclei (cytogenetics) observed in histopathological examinations.

In this study, there was no difference in the animals' body mass in the experimental groups (P> 0.05), like what was found in the study by Piveta (2005).

However, in the global blood count, it was observed that the groups not treated and treated with vehicle had WBC values significantly higher than the CF group. This is in line with what was described by Garcia et al. (2004), who stated that this effect is due to CF, which is a potent immunosuppressant that acts on cells with high mitotic activity, inhibiting the humoral and cellular immune response. Despite treating malignant and non-malignant diseases (Hosseinimehr & Karami, 2005), the side effectsmost commonly observed with CF use refers to the effect on the hematopoietic tissue, causing myelodepression (leukopenia, thrombocytopenia, anemia) (Schuurman et al., 2005), a fact confirmed by the World Health Organization (WHO), which points it out as the most commonly reported adverse reactions in patients undergoing chemotherapy. This adverse reaction is the most important because the hematopoietic tissue has a high rate of cell proliferation (Bonassa & Santana, 2005).

In the relationship between organ weight and body weight, some changes were observed, since the spleen is involved in lymphatic, immunological, circulatory, and hematopoietic functions (Fry & McGavin, 2007). This organ decreased in size in relation to body weight in both groups treated with dynamized drugs and challenged with CF and in the positive control group. In animals treated with CF, there was a reduction in organ weight due to a reduction in extramedullary hematopoiesis, probably due to cell lysis in the parenchyma, leading to a reduction in the red pulp of the organ, which is in accordance with the findings of Schuurman et al. (2005), since hematopoietic tissue has a high rate of cell proliferation

(Bonassa & Santana, 2005). The dynamized drugs did not change the weight of the spleen, which leads to think that these compounds do not affect this function. Fact that which was confirmed by histopathological analysis of the organ, where only animals that received allopathic CF had a degree of injury above 1. Like studies that demonstrated the occurrence of apoptosis in spleens of animals treated with CF (Blankenberg et al., 2001). event that may justify the cellular and splenic reduction founded. However, in the thymus, it is noteworthy that, in addition to the groups not exposed to allopathic cyclophosphamide, the group treated with Cyclophosphamide dynamized intraperitoneally and challenged with Cyclophosphamide showed lower injury graduation averages compared to the control group treated with Cyclophosphamide, apparently partially attenuating the effect of Cyclophosphamide.

Previous studies like that of Eldahshan & Abdel-Daim (2015) have already demonstrated that nux vomica compounds have potent cytotoxic effects against human laryngeal squamous cell carcinoma, breast carcinoma (MCF-7), and cell lines of colon carcinoma), analgesic, antipyretic, and anti-inflammatory activities, and concluded that these activities could be due to the presence of phenolic compounds revealed by our phytochemical investigations. Amaral et al. (2018), in the treatment of tumors in mice with highly diluted thymulin (5CH), observed different biological aspects of the apoptotic activities of tumor cells, although there was no reduction in the macroscopic tumor mass. The immunomodulatory effect of the present study deserves further investigation to assess the real impact, as it was not associated with improvements in body weight, as found in the study by Barbour et al. (2004).

In the Micronucleus Test, the results demonstrated that the frequency of micronuclei in groups of mice that were exposed to the action of the genotoxic agent of Cyclophosphamide is significantly higher in comparison with the negative control group (treated with water), the vehicle and the groups that received only the dynamized compounds. This corroborates with the literature, indicating that CF is a potentially genotoxic substance. In other words, it can produce side effects and tumors due to its genotoxicity (Schuurman et al., 2005; Fenech, 2005). As DNA is the main target of genotoxic agents, these changes can result in cellular dysfunctions, such as genetic instability and mutagenesis (Ferreira, 2008). For this reason, the drug chosen for the present study was positive for control.

It is also noteworthy that, unlike other studies (Banerjee et al., 2007; Biswas and Khuda-Bukhsh, 2002; Nascimento et al., 2016) that showed a reduction in genotoxic capacity by ultra-diluted and dynamized compounds, the same did not occur for nux vomica and CF in the experimental conditions of this study, just as they did not induce genotoxicity, unlike other compounds as already observed for Ruta graveolens and Ruta 200C (Preethi et al., 2008).

Conclusions

The dynamized ultra-diluted compounds of nux vomica and CF, in the formulation used and in the experimental design, showed lower leukocyte counts in mice (Figure 2), similar to the CF group, but without causing damage to the spleen and thymus. Furthermore, the dynamized ultra-diluted CF compound, when used intraperitoneally, appear be able to, at least partially, mitigate the effect of CF on thymic cortical reduction in mice. However, the compounds were not able to mitigate the genotoxic effects of CF and further studies with nux vomica and dynamized CF should be performed to better delineate their possible therapeutic potential in reducing adverse effects on chemotherapy.

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Ethics statement

The study protocol was approved by the FIOCRUZ Animal Ethics Committee, resolution 242/99 (Licensed under number LW16/13 - CEUA/FIOCRUZ).

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Conflict of interests

JSC, MEV, ASR, LFP, ICFB, KCD no conflict of interest, MAAK, no conflict of interest and responsible for the financial support.

Authors' contributions

JSC and MEV- performance of all stages of the experiment. ASR - supply and preparation of homeopathic medication applied in the study. LFP - elaboration of the work. ICFB - critical review and final approval of the version to be published. KCD - responsible for the study, preparation of the work, carrying out all stages of the experiment, data interpretation, critical review and final approval of the version to be published. MAAK - responsible for the study, preparation of the work, carrying out all stages of the experiment and interpreting the data, critical review and final approval of the version to be published.

Availability of complementary results

JJSC, MEV, ASR, LFP, ICFB, KCD, MAAK declare that the complementary information devices as a result of this study are filed in a log book, researchers, at the Cell Biology Laboratory of the Oswaldo Cruz Institute (IOC) of the Foundation Fundação Oswaldo Cruz (Fiocruz).

The study was carried out at Laboratório de Biologia Celular, Instituto Oswaldo Cruz – IOC, Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, RJ, Brasil.

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