## PRISMA 2020 Checklist

| Section and Topic | Item # | Checklist item | Location where item is reported |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Advantages of the use of polymeric nanoparticles in drug delivery compared to the use of free drugs in the treatment of breast cancer: a systematic review | |
| **ABSTRACT** | | | |
| Abstract | 2 | Breast cancer represents a fifth of the total number of patients diagnosed with malignant neoplasms worldwide. Faced with the limitations of conventional treatments, studies are advancing towards developing new therapies with more specificity and fewer side effects. Thus, the use of polymeric nanoformulations for the delivery of chemotherapeutic agents, in which nanoparticles (NPs) perform this delivery in a controlled and more effective way, has been developed for biomedical applications. In this sense, the study aimed to investigate the advantages and effectiveness of the use of polymeric nanoformulations in drug delivery compared to conventional therapies in the treatment of breast cancer. The study is a systematic review that followed the eligibility criteria as established by the acronym PICOS (Population, Intervention, Comparison, Outcome and Study Design), bibliographic searches were performed in January 2022 in PubMed/MEDLINE, Embase and Scopus using specific search strings, totaling 12 articles involving pre-clinical *in vitro* and/or *in vivo* studies, clinical studies and literature review studies. In this systematic analysis, the results showed the effectiveness of the strategy of using polymeric-NPs (NPsP) for drug delivery, with promising results of therapeutic efficacy, highlighting the use of free drugs in the treatment of breast cancer, in which drugs incorporated into the NPs show increased cellular uptake and sustained intracellular retention, which increases the effectiveness of the treatment. However, additional studies are essential to elucidate the effectiveness of this therapeutic strategy. | |
| **INTRODUCTION** | | | |
| Rationale | 3 | A more effective anti-cancer treatment that has fewer side effects for the patient, as the mortality rate from breast cancer has increased dramatically in recent years and is already the most prevalent type of cancer in the world, according to data from the World Organization of Health (WHO, 2021). | |
| Objectives | 4 | This systematic review study aimed to investigate the advantages and efficacy of using polymeric nanoformulations in drug delivery compared to conventional therapies in the treatment of breast cancer. | |
| **METHODS** | | | |
| Eligibility criteria | 5 | The criteria adopted in this systematic review followed the approach of the acronym PICOS (Population, Intervention, Comparison, Outcome and Study Design) (Methley et al., 2014). In which rats, mice and humans, of both sexes, of different ethnicities, diagnosed with breast cancer, seeking alternative medical treatment (P), with drugs incorporated into NPsP (I), compared to treatment with free drugs , chemotherapy, radiotherapy, immunotherapy, among others (C), which have cytotoxic activity and efficacy at the end of treatment, becoming an alternative strategy in the treatment of breast cancer, with less consequences for the patient (O). This study considered review articles and original peer-reviewed articles, published in English (American and British), on NPsP used for drug delivery in the treatment of breast cancer compared to the use of free drugs. The results were organized into sub-themes for a better understanding of the subjects covered in this research, considering a period of searches in the databases of the last 10 years, between 2011 to 2021. | |

<table>
<tr><th>Section and Topic</th><th>Item #</th><th>Checklist item</th><th>Location where item is reported</th></tr>
<tr><td>Information sources</td><td>6</td><td>Scientific prospecting was carried out on January 15, 2022, in PubMed/MEDLINE, Embase and Scopus databases.</td><td></td></tr>
<tr><td>Search strategy</td><td>7</td><td>The scientific survey was carried out on January 15, 2022, in the PubMed/MEDLINE, Embase and Scopus databases using descriptors related to the PICO acronym mentioned in the subsection “Eligibility Criteria” in the Method topic of this study, in which the strings were obtained from queries from specific searches for each database, as available in the table below:
<table>
<tr><th>Bancos de dados</th><th>Strings</th></tr>
<tr><td>PubMed/MEDLINE</td><td>(((((“Humans”[Mesh]) AND “Adult”[Mesh]) AND “Neoplasms”[Mesh]) AND “Drug Delivery Systems”[Mesh]) AND “Polymers”[Mesh]) AND “Nanoparticles”[Mesh]</td></tr>
<tr><td>Embase</td><td>‘human’/exp AND ‘adult’/exp AND ‘breast tumor’/exp AND ‘drug delivery system’/exp AND (‘nanoparticle’/exp AND ‘polymer’/exp OR ‘polymer nanoparticle’/exp)</td></tr>
<tr><td>Scopus</td><td>( TITLE-ABS-KEY ( “homo sapiens*” OR man* OR “modern man*” OR human* ) AND TITLE-ABS-KEY ( “breast neoplasm*” OR “breast tumor*” OR “breast cancer*” ) AND TITLE-ABS-KEY ( pharmaceutical* OR “pharmaceutical preparation*” OR ( preparation* AND pharmaceutical* ) OR drug* ) AND TITLE-ABS-KEY ( ( nanoparticle* AND polymeric* ) OR “polymeric nanoparticle*” OR “nanosized particle polymeric*” ) AND TITLE-ABS-KEY ( therap* OR drug* OR “drug therap*” OR “drug 2harmacother*” OR 2harmacother* OR 2harmacotherapy* ) AND TITLE-ABS-KEY ( adult* ))</td></tr>
</table></td><td></td></tr>
<tr><td>Selection process</td><td>8</td><td>The criteria adopted in this systematic review followed the approach of the acronym PICOS (Population, Intervention, Comparison, Outcome and Study Design) (Methley et al., 2014). In which rats, mice and humans, of both sexes, of different ethnicities, diagnosed with breast cancer, seek alternative medical treatment (P), with drugs incorporated into the NPsP (I), compared to treatment with free drugs, chemotherapy, radiotherapy , immunotherapy , among others (C), which have cytotoxic activity and efficacy at the end of treatment, becoming an alternative strategy in the treatment of breast cancer, with fewer</td><td></td></tr>
</table>

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| | | consequences for the patient (O). This study considered review articles and original peer-reviewed articles, published in English (American and British), on NPsP used for drug delivery in the treatment of breast cancer compared to the use of over-the-counter drugs. The results were organized into sub-themes for a better understanding of the subjects addressed in this research, considering a period of searches in the databases of the last 10 years, between 2011 to 2021. | |
| Data collection process | 9 | After bibliographic searches in the databases, the articles that were used in this study were selected. Thus, the articles were screened in two stages and, in the first stage, four reviewers (Vieira, J. A; Araújo, J. L; dos Santos Silva, M; da Silva Santos, A. B) independently evaluated all the articles extracted from the databases taking into account the eligibility criteria established in this study. Then, in the second stage, the full texts of each article were obtained and evaluated by reading in full and selecting the articles relevant to the pre-defined themes and/or sub-themes. Subsequently, the diagramming of the obtained data was organized in a section of "Results and Discussion" written by the six reviewers of this study (Vieira, J. A; Araújo, J. L; dos Santos Silva, M; da Silva Santos, AB ; da Silva Luz, G. V; and Azevedo, R. B) strictly following the PRISMA 2020 verification protocol for a systematic review study (Moher et al., 2009; Page et al., 2021). | |
| Data items | 10a | Bibliographic searches in the databases were carried out, and a total of 52 scientific articles were found, in which only 25 studies remained after removing the duplicates, which were carried out by automation with the Mendeley Desktop 2.61.1 software. Thus, we obtained a total of 11 scientific articles in the Embase database, 8 in PubMed/MEDLINE and 6 in Scopus. After analyzing the inclusion and exclusion of articles based on the eligibility criteria established in this study, 12 articles were selected for this systematic review. Of these, 7 are pre-clinical in vitro and/or in vivo studies, 4 clinical studies and 1 literature review studies. It is worth mentioning that of the 52 articles found, only 1 article presented a duplicate in the Scopus database and 26 articles in the Embase database from which they were taken. Therefore, the criteria established in the acronym PICO were essential for the selection of articles and removal of terms in the DECs and MeSh bases, for the construction of specific search strings that were applied to extract the data in each bibliographic base, bringing greater reliability to this study. study. In additiontocheckingthe PRISMA 2020 checklist. | |
| | 10b | **Nanoformulation type:** Only NPsP, PM and other polymeric nanoformulations were included; **Co-delivery:** Capacity of the NPsP or PM to deliver the active ingredient; **Toxicity:** Nanoformulations that showed low toxicity in their clinical or pre-clinical analyses; **Efficacy:** Nanoformations that showed efficacy in inhibiting breast cancer tumor cells. | |
| Study risk of bias assessment | 11 | The studies collected in this systematic review showed methodological heterogeneity in the conduct of each research. Wherein, they ranged from pre-clinical studies to randomized and non-randomized clinical trials. Thus, we evaluated the quality of the studies, in which 2 independent reviewers (Vieira, J. A and Araújo, J. L) assessed the risk of bias of the articles included through the application of a questionnaire (which is available in Table S1 of the list of PRISMA 2020 verification in the supplementary material) and submitted to the RoB 2.0 tool (Cochrane Revised Risk of Bias Tool for Randomized Trials) (Sterne et al., 2019), which assessed the quality and bias of randomized controlled trials and the ROBINS tool -I (Risk Of Bias In Non-randomized Studies - of Interventions) (Sterne et al., 2016) for non-randomized clinical trials. In addition, we used the "Generic" tool to assess the quality of preclinical studies (in vitro, ex vivo and in vivo). It is worth mentioning that all these tools are part of the Robvis (Risk-of-bias VISualization) software package (Mcguinness; Higgins, 2021). In case of disagreements between the first two reviewers, a third reviewer (dos Santos Silva, M) was requested. The Bias Risk Tools are based on 05 domains, namely: | |

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| | | 1) Bias resulting from the randomization process; 2) Bias due to deviations from intended interventions; 3) Bias due to lack of results data; 4) Outcome measurement bias and 5) Reported study selection bias (Higgins et al., 2019). | |
| Effect measures | 12 | The assessment of the quality and risk of bias of the studies discussed in this systematic review was performed according to the method presented in each study. In which randomized clinical trials were evaluated and defined as: low risk of bias, not informed, some concerns or high risk of bias, using the RoB 2.0 tool, in which the algorithm judged the risk of bias of the articles Ranade et al. (2013) and Fujiwara et al. (2019), based on the questions in each domain. Therefore, it was observed that the two randomized clinical trials were classified as low risk of bias and points to a high quality of studies for all 5 domains, in addition, the articles are classified at 100% as low risk of bias, increasing the reliability of the results presented in this review. For non-randomized clinical trials, we observed that domain 2 for the study by Gaur et al. (2014) was classified as "Moderate" and in the study by Shi M et al. (2017) in domain 6 as "Serious", as illustrated in Figure 4a. However, we observed that it did not compromise the quality of the studies in the overall assessment and that the risk of bias was considered low for both articles. However, we highlight that there is a 50% chance that there is a serious risk of bias for domain 6 "Bias in the measurement of results". In the evaluation of pre-clinical studies, the "Generic" tool of the Robvis software (Mcguinness; Higgins, 2021) showed that the study by Fulfager& Yadav (2021) has a high risk of bias, which makes perfect sense, as the authors investigated the co-delivery of therapeutic agents in a nanocarrier to combat multidrug resistance (MDR) in breast cancer through an integrative review study. Thus, the software pointed to domains 2 and 5 "not clear" and to domain 4 "severe risk of bias".<br>Furthermore, we observed that all preclinical studies discussed in this systematic review are of high quality for domains 1, 3 and 7, presenting "low risk of bias" in up to 100% of their results. We also highlight that there is a 45% chance of studies not presenting clarity regarding domain 2 and a rate of 10% for domains 4, 5 and 6, respectively. In addition, there is a "severe risk of bias" of 10% for domains 4 and 6, in addition to a lack of information of up to 55% for domain 2. | |
| Synthesis methods | 13a | The criteria adopted in this systematic review followed the approach of the acronym PICOS (Population, Intervention, Comparison, Outcome and Study Design) (Methley et al., 2014). In which rats, mice and humans, of both sexes, of different ethnicities, diagnosed with breast cancer, seeking alternative medical treatment (P), with drugs incorporated into NPsP (I), compared to treatment with free drugs, chemotherapy, radiotherapy, immunotherapy, among others (C), which have cytotoxic activity and efficacy at the end of treatment, becoming an alternative strategy in the treatment of breast cancer, with less consequences for the patient (O). | |
| | 13b | All the extracted files were computed in the Mendeley Desktop 2.61.1 software, which was used to remove duplicates by automation. In addition to these tools, we used the freely available VOSviewer 1.6.8 software (https://www.vosviewer.com/) to assess citation relationship networks, bibliographic coupling and co-citations related to the descriptors of this study. | |
| | 13c | After the bibliographic searches in the databases, the data of the articles found were tabulated in an Excel spreadsheet (Microsoft Excel tool (2007)) for the development of the analyses. In addition to these tools, we used the freely available VOSviewer 1.6.8 software (https://www.vosviewer.com/) to assess citation relationship networks, bibliographic coupling and co-citations related to the descriptors of this study. | |

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| | 13d | To summarize the results, we started by creating a table with the strings used in the searches by base. We also made a graph that has the number of studies per base. Then, the prism diagram (2020), because in it we can visualize the steps that the study went through: number of databases, number of duplicates removed, removal after the first filter, removal after the second filter, eligibility application, excluded studies and included studies. The keyword network of the search findings (n = 25) was analyzed in the VOSviewer 1.6.8 software. The different sizes of the nodes represent the number of times each word is cited, the links refer to co-citations. After these analyses, we created a table of characteristics that encompasses all the studies included in the review and finally we present figures that evaluated the quality of the studies using Rob 2.0 and ROBINS-I software. | |
| | 13e | Filter 2 described in the methods of this study was used to identify possible causes of heterogeneity between articles. | |
| | 13f | At the end of data collection, analyzes were performed using Robvis software (Risk-of-bias VISualization), in order to compare the robustness of the results. | |
| Reporting bias assessment | 14 | The studies collected in this systematic review showed methodological heterogeneity in the conduct of each research. In which, they ranged from pre-clinical studies to randomized and non-randomized clinical trials. Thus, we evaluated the quality of the studies, in which 2 independent reviewers (Vieira, J. A and Araújo, J. L) assessed the risk of bias of the articles included through the application of a questionnaire (which is available in Table S1 of the PRISMA 2020 checklist in the supplementary material) and submitted to the RoB 2.0 tool (Revised Cochrane risk-of-bias tool for randomized trials) (Sterne et al., 2019), which evaluated the quality and bias of randomized clinical trials and the ROBINS tool -I (Risk Of Bias In Non-randomised Studies - of Interventions) (Sterne et al., 2016) for non-randomized clinical trials. In addition, we use the "Generic" tool to assess the quality of preclinical studies (*in vitro*, *ex vivo* and *in vivo*). It is worth noting that all these tools are part of the Robvis (Risk-of-bias VISualization) software package (Mcguinness; Higgins, 2021). In case of disagreements between the first two reviewers, a third reviewer (dos Santos Silva, M) was requested.<br>The Risk of Bias Tools are based on 05 domains, namely: 1) Bias resulting from the randomization process; 2) Bias due to deviations from intended interventions; 3) Bias due to lack of results data; 4) Bias in outcome measurement and 5) Bias in the selection of the reported study (Higgins et al., 2019). | |
| Certainty assessment | 15 | The RoB 2.0, ROBINS-I and Generic tools were used to assess the quality of the articles, as well as the risk of bias, which measures the reliability of each study. | |
| **RESULTS** | | | |

# PRISMA 2020 Checklist

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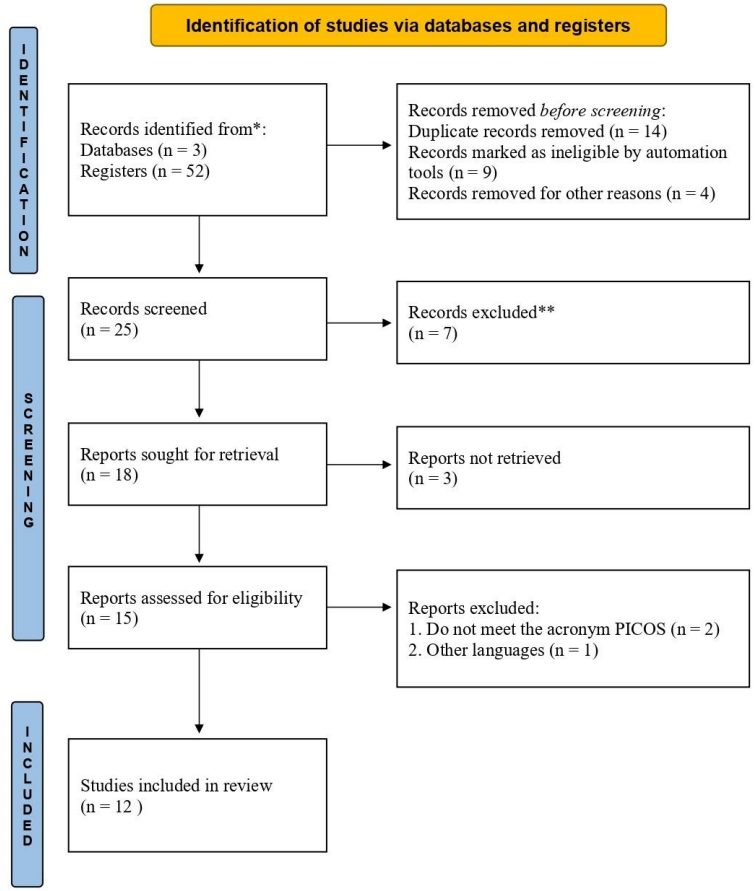
| Study selection | 16a |  | |

<table>
<tr><th>Section and Topic</th><th>Item #</th><th>Checklist item</th><th>Location where item is reported</th></tr>
<tr><td></td><td>16b</td><td>
<table>
<tr><th>Authors</th><th>Name</th><th>Year</th><th>Exclusion reason</th></tr>
<tr><td>Gradishar, W. J., et al. Journal of Clinical Oncology.</td><td>Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer.</td><td>2005</td><td>Year</td></tr>
<tr><td>Nyman, D. W., et al. Journal of Clinical Oncology.</td><td>Phase I and pharmacokinetics trial of ABI-007, a novel nanoparticle formulation of paclitaxel in patients with advanced nonhematologic malignancies.</td><td>2005</td><td>Year</td></tr>
<tr><td>Paolini, M., et al. International Journal of Nanomedicine.</td><td>Nano-sized cytochrome P450 3A4 inhibitors to block hepatic metabolism of docetaxel.</td><td>2017</td><td>Target cells</td></tr>
</table>
</td><td></td></tr>
<tr><td>Study characteristics</td><td>17</td><td>
<table>
<tr><th>Author</th><th>Type of NPs</th><th>Drugs</th><th>Studt type</th><th>Time</th><th>Efficiency</th><th>Toxicity</th></tr>
<tr><td>Ranade, A. A., et al. Asia Pac J Clin Oncol. (2013)</td><td>PM</td><td>PTX in different concentrations</td><td>Randomized clinical trial</td><td>3 weeks</td><td>Showed better safety and promising efficacy in patients with advanced and/or metastatic breast cancer</td><td>Anemia, Neutropenia, Leukopenia, Oral mucositis and Diarrhea Neuropathy</td></tr>
<tr><td>Fulfager, A., & Yadav, K. S. J. drug del. sci. tech. (2021)</td><td>Liposomes, PM, NPsP, Metallic Lipid Dendrimers</td><td>Adriamycin, Quercetin, PTX, Rapamycin, Doxorubicin, Verapamil, Imatinib, Retinoic Acid, Curcumin, DTX, Chloroquine, DOX, PDTC, BCL, PDA FA, Methoxy-</td><td>Preclinical in vitro and in vivo</td><td>N/A</td><td>Aid in drug pharmacokinetics, prevent drug degradation, controlled release and reduced side effects</td><td>May reduce chemotherapy toxicity</td></tr>
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| Section and Topic | Item # | Checklist item | | | | | | | | Location where item is reported |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | poly(ethylene glycol)-b-poly((2-dimethylamino)ethyl methacrylate - itaconic acid) (mPEG-b-p (DMAEMA-co-IA)) copolymer. Salinomycin, Pluronic, Lapatinib, Elacridar (GG918), Cyclosporine A, Lonidamine and Resveratrol | | | | | |
| | | | Oda, C. M. R., et al. *J Drug Deliv Sci Technol*. (2018) | PM containing PTX (PM-DTPA/PTX) | PTX (PM-DTPA/PTX) | Preclinical *in vitro* and *in vivo* | 24 h | The lyophilized kit was able to protect and maintain the physicochemical and radiochemical pharmacological properties of PM-DTPA/PTX until delivery to 4T1 murine mammary carcinoma cells | N/A | |
| | | | Khan, M. A., et al. *Int. J. Biol. Macromol*. (2017) | Chitosan with Carboplatin | carboplatin | Preclinical *in vitro* and *in vivo* | 24, 48 e 73 h | The chitosan NPs showed better performance than the free drug both in delivery and in its therapeutic potential. $IC_{50}$ value for 24 h (120.23 µg/mL), 48h (94.17 µg/mL) and 72 h | Colloidal positively charged particles can enhance the ionic interaction between negatively charged particles on the mucosal surface. It is improving its bioavailability and decreasing its toxicity. | |

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| | | | | | | | (86.25 µg/mL), respectively. The values were higher compared to those obtained with free carboplatin | | |
| | | | Chidambaram, M., & Krishnasamy, K. *Nano Biomed. Eng.* (2014) | Curcumin polymers | Cur-Pi, Cur-Qu e Cur-Si. | Preclinical *in vitro* and *in vivo* | 31 days | In vitro cytotoxicity by MTT assays showed that the nanoformulations had a lower $IC_{50}$ than pure curcumin. In *in vivo* assays, nanoformulations had their biological activities increased compared to pure curcumin | They did not induce significant changes in cells that remained within normal histopathological limits in anatomopathological examinations of vital organs such as the liver, kidney, heart and brain | |
| | | | Yap, Jeaniffer E. et al. *Macromol. Rapid Commun.* (2020) | PM DASA | Ellipticine | Preclinical *in vitro* | N/A | DASA polymers encapsulated ellipticin and the drug was successfully translocated into MCF-7 breast cancer cells by micelles. | Drug loaded micelles are more toxic after 2 h irradiation compared to the non-irradiated sample and the irradiated sample increased toxicity after 72 h | |

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| | | Xiao, K., et al. *Adv. Healthc. Mater*. (2020) | PM (PEG) and Cholic Acid (CA) | Disorazol Z and DOX (PTX-LHRH-DCMs) | Preclinical *in vitro*, *in vivo* and *ex vivo* | 24 days | NP-achieved high efficacy in loading, releasing and antitumor response, reducing systemic toxicity | NPs do not exhibit hematologic, hepatic and renal toxicity | |
| | | Gaur, S., et al. *Nanomedicine*, (2014) | Drug-based polymer (DBP) | Cyclodextrin and Camptothecin (CRLX101) | Preclinical *in vitro* and clinical study | 4 weeks | The therapy proved to be effective against some types of tumors such as breast, prostate and pancreas | Gastrointestinal toxicity, hemorrhage, cystitis, and severe bone marrow suppression | |
| | | Manjili, H. K., et al. *Artif Cell Nanomed B.* (2017) | PM (PCL/PEG/PCL) | Artemisinin (ART) | Preclinical *in vitro* and *in vivo* | 4 weeks | High therapeutic and encapsulation efficacy of ART-PCL-PEG-PCL micelles | The results of this evaluation do not show toxicity to naked nanoparticles at various concentrations. Relative cell toxicity was calculated by: relative cell toxicity = $[(A_{sample} - A_{control}) / Acontrol] X 100$ | |
| | | Fujiwara, Y., et al. *Br. J. Cancer*. (2019) | PM (NK105) | PTX | Randomized clinical Trial | 28 days | Little difference comparing the use of the formulation (NK105) with drug (PTX) in the treatment | Grade 3 infections, febrile neutropenia, and severe thrombocytopenia | |
| | | Abou-El-Naga A. M., et al. *Int J Nanomedicine*. (2018) | Polymeric NPs based on poly(lactic acid-co-glycolic acid) PLGA | DTX | Preclinical *in vitro* | 2 weeks | NPs (PLGA-DTX) have been shown to be a promising option | N/A | |

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| | | | | | | | in the treatment of breast câncer | | |
| | | Shi M., et al. *Invest New Drugs*, (2017) | PM with PTX | PTX | Clinical study | 1 year | Clinical trials have shown that the formulation used has antitumor and tumor size reduction efficacy | Haematological/non-haematological effects were transient, included anemias and skin reactions | |
| Risk of bias in studies | 18 | **Figure 1.** Risk of bias of randomized clinical trials included and evaluated through RoB 2.0. | | | | | | | |

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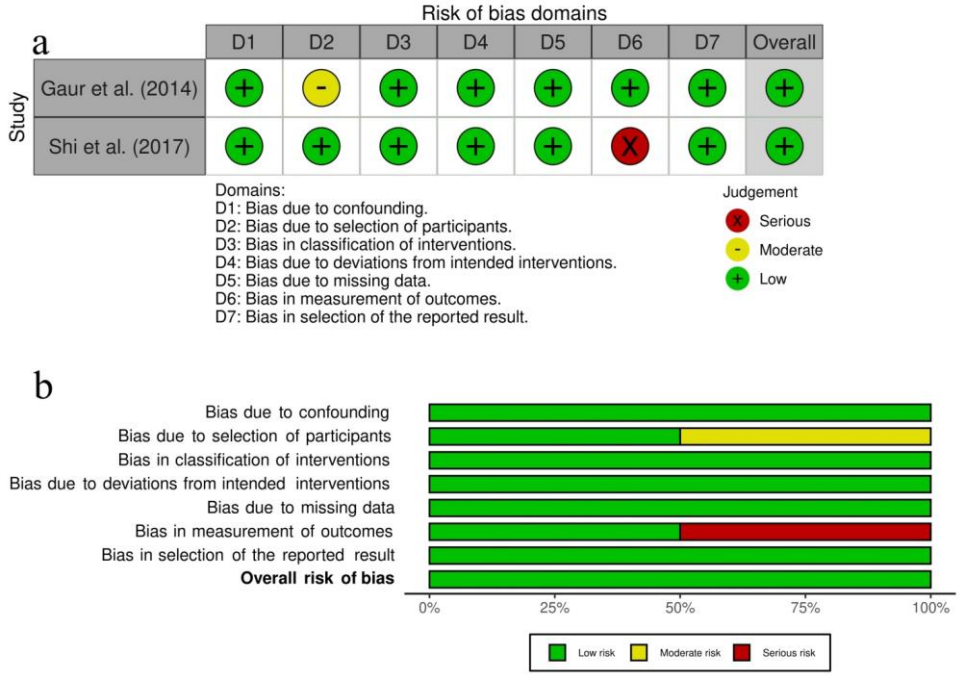
| | |  <br>**Figure 2.** Risk of bias from non-randomized clinical trials included and assessed using the ROBINS-I. | |

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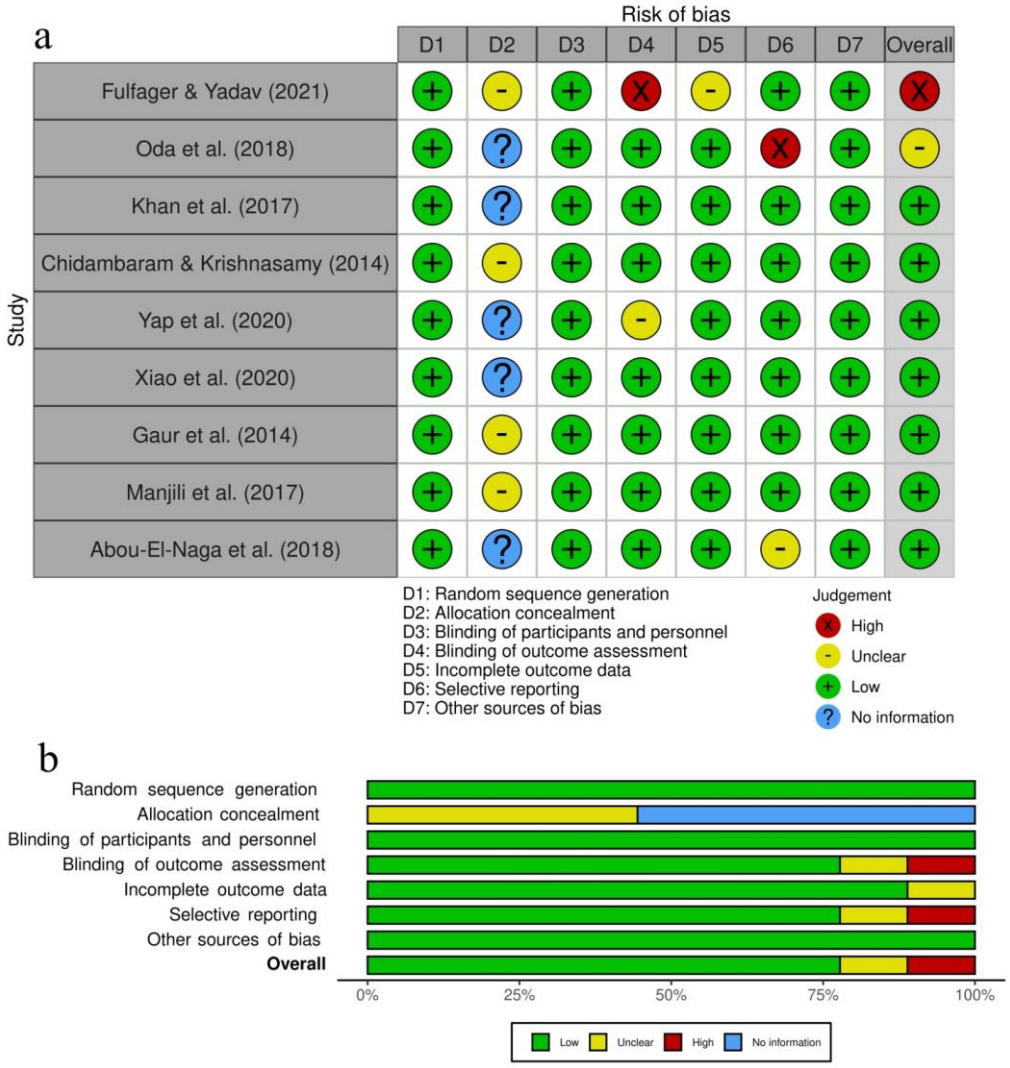
| | |   **Figure 3.** Risk of bias from preclinical studies included and evaluated using GENERIC. | |
| Results of individual | 19 | **Article** \| **Quality assessment (confidence)** \| **Risk of bias characteristic** \| **Overall** | |

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| studies | | Gaur et al. (2014) | ROBINS-I | There was "moderate" risk in the D2 domain (Bias due to selection of participants) and in the other domains there was a low risk of bias. | Low | |
| | | Shi et al. (2017) | ROBINS-I | There was "serious" risk in the D6 domain (Bias in measurement of outcomes) and in the other domains there was a low risk of bias. | Low | |
| | | Ranade et al. (2013) | RoB 2.0 | All domains had a low risk of bias. | Low | |
| | | Fujiwara et al. (2019) | RoB 2.0 | all domains had a low risk of bias. | Low | |
| | | Fulfager & Yadav (2021) | Generic | There was "unclear" risk in the D2 domain (Allocation concealment) and D5 domain (Incomplete outcome data). Domain D4 (Blinding of outcome assessment) had a high risk of bias and in the other domains there was a low risk of bias. | High | |
| | | Oda et al. (2018) | Generic | There was "no information" in D2 domain (Allocation concealment). There was "high" risk in the D6 domain (Selective reporting) and in the other domains there was a low risk of bias. | Unclear | |
| | | Khan et al. (2017) | Generic | There was "no information" in D2 domain (Allocation concealment) and in the other domains there was a low risk of bias. | Low | |
| | | Chidambaram &Krishnasamy (2014) | Generic | There was "unclear" risk in the D2 domain (Allocation concealment) and in the other domains there was a low risk of bias. | Low | |
| | | Yap et al. (2020) | Generic | There was "no information" in D2 domain (Allocation concealment). There was "unclear" risk in the D4 domain (Blinding of outcome assessment) and in the other domains there was a low risk of bias. | Low | |
| | | Xiao et al. (2020) | Generic | There was "no information" in D2 domain (Allocation concealment) and in the other domains there was a low risk of bias | Low | |

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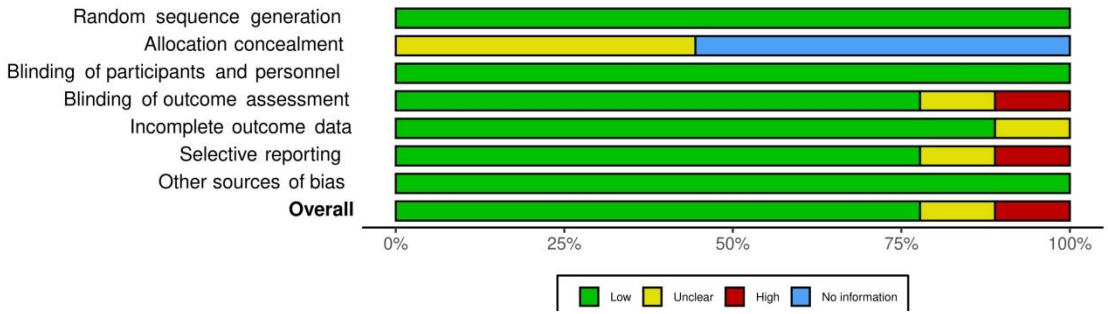
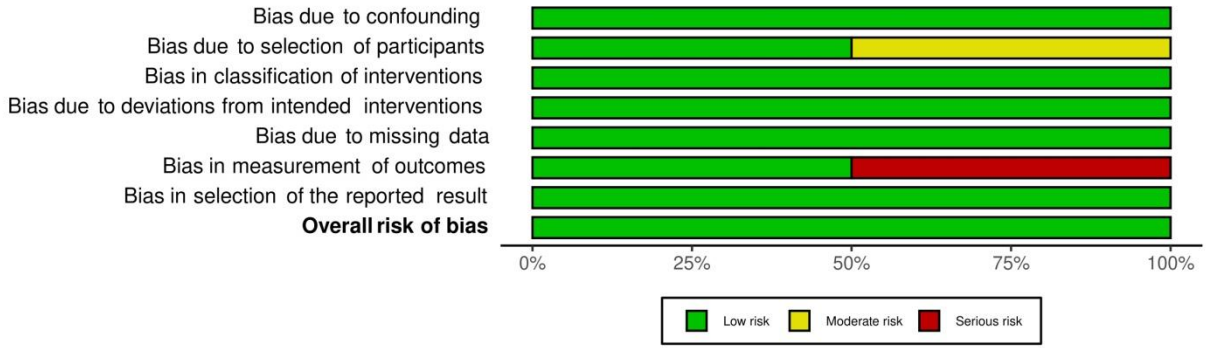
<tr><td></td><td></td><td>
<table>
<tr><td>Gaur et al. (2014)</td><td>Generic</td><td>There was “unclear” risk in the D2 domain (Allocation concealment) and in the other domains there was a low risk of bias.</td><td>Low</td></tr>
<tr><td>Manjili et al. (2017)</td><td>Generic</td><td>There was “unclear” risk in the D2 domain (Allocation concealment) and in the other domains there was a low risk of bias</td><td>Low</td></tr>
<tr><td>Abou-El-Naga et al. (2018)</td><td>Generic</td><td>There was “no information” in D2 domain (Allocation concealment). There was “unclear” risk in the D6 domain (Selective reporting) and in the other domains there was a low risk of bias</td><td>Low</td></tr>
</table>
</td><td></td></tr>
<tr><td>Results of syntheses</td><td>20a</td><td>Gaur et al. (2014) - there was “Moderate” risk in the D2 domain (Bias due to selection of participants)and in the other domains there was a low risk of bias.<br>Shi et al. (2017) - there was “Serious” risk in the D6 domain (Bias in measurement of outcomes)and in the other domains there was a low risk of bias.<br>Ranade et al. (2013) - all domains had a low risk of bias.<br>Fujiwara et al. (2019) - all domains had a low risk of bias.<br>Fulfager& Yadav (2021) - there was “Unclear” risk in the D2 domain (Allocation concealment)and D5 domain (Incomplete outcome data). Domain D4 (Blinding of outcome assessment) had a “High” risk of biasand in the other domains there was a low risk of bias.<br>Oda et al. (2018) - there was “No information” in D2 domain (Allocation concealment). There was “High” risk in the D6 domain (Selective reporting) and in the other domains there was a low risk of bias.<br>Khan et al. (2017) - there was “No information” in D2 domain (Allocation concealment)and in the other domains there was a low risk of bias.<br>Chidambaram & Krishnasamy (2014) - there was “Unclear” risk in the D2 domain (Allocation concealment)and in the other domains there was a low risk of bias.<br>Yap et al. (2020) - there was “No information” in D2 domain (Allocation concealment). There was “Unclear” risk in the D4 domain (Blinding of outcome assessment)and in the other domains there was a low risk of bias.<br>Xiao et al. (2020) - there was “No information” in D2 domain (Allocation concealment)and in the other domains there was a low risk of bias.<br>Gaur et al. (2014) - there was “Unclear” risk in the D2 domain (Allocation concealment) and in the other domains there was a low risk of bias.<br>Manjili et al. (2017) - there was “Unclear” risk in the D2 domain (Allocation concealment) and in the other domains there was a low risk of bias.<br>Abou-El-Naga et al. (2018) - there was “No information” in D2 domain (Allocation concealment). There was “Unclear” risk in the D6 domain (Selective reporting) and in the other domains there was a low risk of bias.</td><td></td></tr>
<tr><td></td><td>20b</td><td>Statistical study carried out by the GENERIC tool:</td><td></td></tr>
</table>

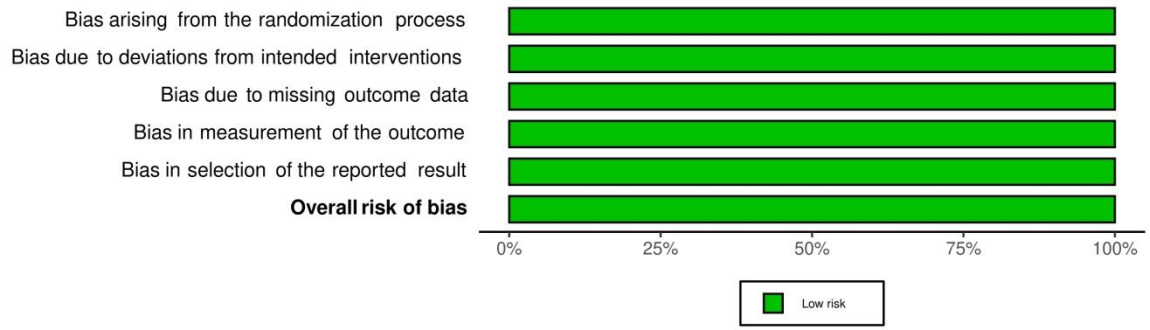
| Section and Topic | Item # | Checklist item | Location where item is reported |
|---|---|---|---|

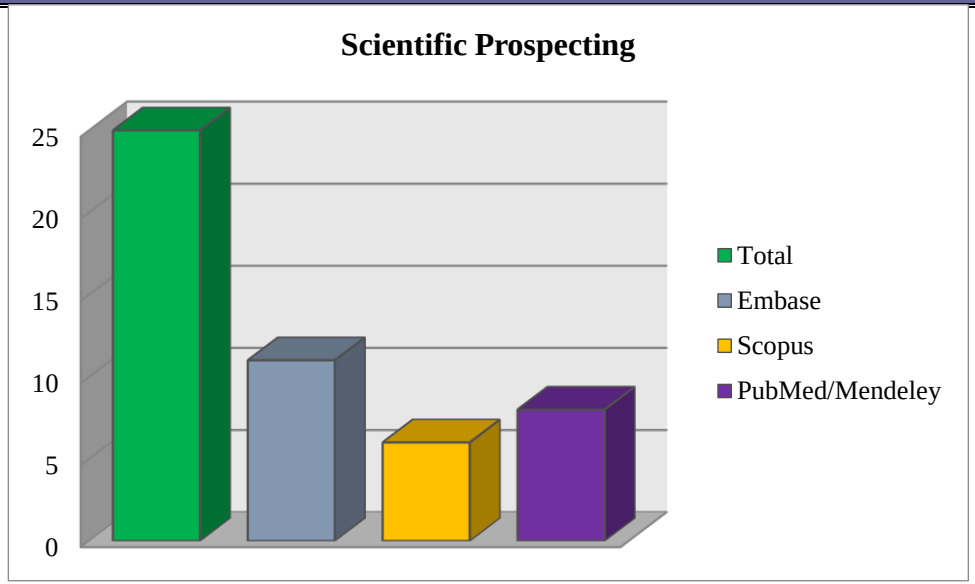



Statistical study carried out by the ROBINS-I tool:




Statistical study carried out by the RoB 2.0 tool:

| Section and Topic | Item # | Checklist item | Location where item is reported |
|---|---|---|---|
| | |  | |
| | 20c | The bibliographic searches in the databases were successfully carried out, with a total of 52 scientific articles being found, in which only 25 studies remained after removing the duplicates, which were performed by automation using the *Mendeley Desktop* 2.61.1 software. Thus, we obtained a total of 11 scientific articles in the Embase database, 8 in PubMed/MEDLINE and 6 in Scopus (Graph 1). After analyzing the inclusion and exclusion of articles based on the eligibility criteria established in this study, 12 articles were selected for this systematic review (Table 2). Of these, 7 are pre-clinical *in vitro* and/or *in vivo* studies, 4 clinical studies and 1 literature review studies, as available in Table 2It is worth mentioning that of the 52 articles found, only 1 article presented a duplicate in the Scopus database and 26 articles in the Embase database from which they were removed. | |

| Section and Topic | Item # | Checklist item | Location where item is reported |
|---|---|---|---|
| | |  **Graph 1.** Number of articles found in the databases. (Analysis in Microsoft Excel (2007)). **Source:** Own authorship (2022).<br><br>Therefore, the criteria established in the PICO acronym were essential for the selection of articles and removal of terms in the DECs and MeSh bases, for the construction of specific search strings that were applied to extract the data in each bibliographic database, bringing greater reliability and basis for this study. | |
| | 20d | We observed that the two randomized clinical studies were classified as low risk of bias and points to a high quality of studies for all 5 domains (Figure 3a), in addition, in Figure 3b we see that the articles are classified at 100% as low risk of bias, increasing the reliability of the results presented in this review. For non-randomized clinical trials, we observed that domain 2 for the study by Gaur et al. (2014) was classified as "Moderate" and in the study by Shi M et al. (2017) in domain 6 as "Serious", as illustrated in Figure 4a. However, we observed that it did not compromise the quality of the studies in the overall assessment and that the risk of bias was considered low for both articles. However, we highlight that there is a 50% chance that there is a serious risk of bias for domain 6 "Bias in measurement of outcomes", as seen in Figure 4b. In the evaluation of preclinical studies, the "GENERIC" tool of the Robvis software (Mcguinness; Higgins, 2021) showed that the study by Fulfager& Yadav (2021) has a high risk of bias (Figure 5), which makes perfect sense, as the authors investigated the co-delivery of therapeutic agents in a nanocarrier to combat multidrug resistance (MDR) in breast cancer through an integrative review study. Thus, the software pointed to domains 2 and 5 "not clear" and to domain 4 "serious risk of bias", as we can see in Figure 5a.In addition, we observed that all preclinical studies discussed in this systematic review are of high quality for domains 1, 3 and 7, presenting "low risk of bias" in up to 100% | |

<table>
<tr><th>Section and Topic</th><th>Item #</th><th>Checklist item</th><th>Location where item is reported</th></tr>
<tr><td></td><td></td><td>of their results, as shown in Figure 5b. We also highlight that there is a chance of 45% of the studies not presenting clarity regarding domain 2 and a rate of 10% for domains 4, 5 and 6, respectively. In addition, there is a 10% “serious risk of bias” for domains 4 and 6, in addition to a lack of information of up to 55% for domain 2, as shown in Figure 5b.</td><td></td></tr>
<tr><td>Reporting biases</td><td>21</td><td>The articles that presented missing results through the risk of bias assessment are contained in the table below:<br><br>
<table>
<tr><th>Article</th><th>Domains</th><th>Judgements</th></tr>
<tr><td>Shi et al. (2017)</td><td>Bias in measurement of outcomes</td><td>High</td></tr>
<tr><td>Fulfager& Yadav (2021)</td><td>Blinding of outcome assessment</td><td>High</td></tr>
<tr><td>Oda et al. (2018)</td><td>Allocation concealment/Selective reporting</td><td>No information/High</td></tr>
<tr><td>Khan et al. (2017)</td><td>Allocation concealment</td><td>No information</td></tr>
<tr><td>Yap et al. (2020)</td><td>Allocation concealment</td><td>No information</td></tr>
<tr><td>Xiao et al. (2020)</td><td>Allocation concealment</td><td>No information</td></tr>
<tr><td>Abou-El-Naga et al. (2018)</td><td>Allocation concealment</td><td>No information</td></tr>
</table></td><td></td></tr>
<tr><td>Certainty of evidence</td><td>22</td><td>Robvis online software was used to assess the quality (confidence) of each study, as shown in the table below:<br><br>
<table>
<tr><th>Article</th><th>Quality assessment (confidence)</th></tr>
<tr><td>Gaur et al. (2014)</td><td>ROBINS-I</td></tr>
<tr><td>Shi et al. (2017)</td><td>ROBINS-I</td></tr>
<tr><td>Ranade et al. (2013)</td><td>RoB 2.0</td></tr>
<tr><td>Fujiwara et al. (2019)</td><td>RoB 2.0</td></tr>
<tr><td>Fulfager& Yadav (2021)</td><td>GENERIC</td></tr>
<tr><td>Oda et al. (2018)</td><td>GENERIC</td></tr>
<tr><td>Khan et al. (2017)</td><td>GENERIC</td></tr>
<tr><td>Chidambaram &Krishnasamy (2014)</td><td>GENERIC</td></tr>
<tr><td>Yap et al. (2020)</td><td>GENERIC</td></tr>
<tr><td>Xiao et al. (2020)</td><td>GENERIC</td></tr>
<tr><td>Gaur et al. (2014)</td><td>GENERIC</td></tr>
<tr><td>Manjili et al. (2017)</td><td>GENERIC</td></tr>
<tr><td>Abou-El-Naga et al. (2018)</td><td>GENERIC</td></tr>
</table></td><td></td></tr>
<tr><td colspan="3">DISCUSSION</td><td></td></tr>
</table>

| Section and Topic | Item # | Checklist item | Location where item is reported |
|---|---|---|---|
| Discussion | 23a | Breast cancer has been the subject of several studies, because depending on its prognosis and stage of the disease, conventional therapies such as chemotherapy, radiotherapy and surgeries may be ineffective, in addition to being invasive treatments and presenting high toxicity causing several side effects and/or adverse effects to the patient. Thus, the applicability of polymeric nanoformulations as carriers of antitumor drugs, based on the literature of this systematic review, demonstrated great therapeutic potential for the treatment of breast cancer, that is, a more effective, controlled and targeted delivery to the desired target was observed.<br>In addition, we can evidence in studies developed by Abou-El-Naga et al. (2018), in which they performed *in vitro* assays using NPsP loaded with a drug that could be less toxic and more assertive in the treatment of breast cancer. For this, NPsP based on PLGA or poly (L-lactic acid-co-glycolic acid) was used, which is a copolymer used in a wide variety of therapeutic mechanisms, precisely because of its biocompatibility and biodegradability characteristics. In conjunction with the drug Docetaxel (DTX), which is used in the antineoplastic treatment of various types of cancers, such as lung, prostate, head, neck, gastric, ovary, bladder and especially breast cancer. Tests showed that the drug DTX was effectively encapsulated in NPsP. The MTT experiments lasted 2 weeks and showed that drug uptake depended on the time cells were exposed to it, reaching its maximum after activation of the endocytosis mechanism, which occurs when living cells absorb some material or substance through the cell membrane. It was also observed that cytotoxicity depends on the concentration used, and that the use of the PLGA-DTX system generates a greater apoptotic effect (which would be programmed cell death) through the activation of Caspase-9, Caspase-3 and P53 genes. Therefore, the proposed NPsP demonstrated a promising drug delivery system in the treatment of breast cancer. | |
| | 23b | In preparing this systematic review, some limitations were noted. First, there was heterogeneity in the analysis of the methodologies of each study, characteristics of NPsP, brands of chemical products, different formulation methods. Thus, we submit the studies to a quality assessment and bias through the online software Robvis. | |
| | 23c | Some limitations were used in the review process, such as characteristics of NPsP or polymeric nanoformulations, stability of nanoformulations, in addition to heterogeneity in the methodologies used in each study. | |
| | 23d | The systematic argument of the bibliographic searches of this study was obtained from the analysis of 12 studies that bring together pre-clinical results *in vitro*, *ex-vivo* and *in vivo* (mice and rats) and clinical trials (patients with confirmed diagnosis of breast cancer and/or relapsed patients), which indicate the effectiveness of the use of polymeric nanoformulations for the delivery of drugs to the target site in the treatment of breast cancer, in which this strategy improved the performance of the drug, having a greater concentration of the active ingredient in the tissues cancer patients, in addition to the increase in drug uptake and cellular concentration, as well as in sustaining their intracellular retention, which increases the effectiveness of the treatment, due to its prolongation linked to the slow release and in a smaller amount of the drug. In this sense, it was also observed that NPsP decreased the side effects, considering that the active ingredient of the drug remains longer in the cancerous tissue, so smaller amounts of antitumor drugs are administered to the patient, offering a concentrated delivery in the affected area long-term. However, additional clinical studies are essential to confirm the use of polymeric nanoformulations in drug delivery in the treatment of breast cancer. | |
| **OTHER INFORMATION** | | | |

## PRISMA 2020 Checklist

| Section and Topic | Item # | Checklist item | Location where item is reported |
|---|---|---|---|
| Registration and protocol | 24a | This systematic review study received deposit approval in the Prospective Register of Systematic Reviews (PROSPERO) under No. (XXXX) (https://www.crd.york.ac.uk/PROSPERO/). This platform is a systematic review study registry base, in which the research registry promotes transparency and eligibility in the execution of the study. In addition, it aims to document systematic reviews already developed or in progress, avoiding the simultaneous execution of research on the same topic by different groups, which prevents and monitors plagiarism. | |
| | 24b | The protocol can be accessed from the Prospective Register of Systematic Reviews (PROSPERO), available from: XXXXXXXXX | |
| | 24c | There was no change between the article and the protocol deposited in the PROSPERO system. | |
| Support | 25 | This study did not receive any public or private financial support. | |
| Competing interests | 26 | The authors declare no conflicts of interest regarding the publication of this paper. | |
| Availability of data, code and other materials | 27 | The entire protocol of this systematic review can be accessed on the PROSPERO platform, available from: XXXXXXXXXXXXX. | |

*From:* Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71.doi: 10.1136/bmj.n71

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