PEER REVIEW, Vol. 6, Nº 10, 2024
DOI: 10.53660/PRW-2189-4028
ISSN: 1541-1389

A systematic literature review on the pharmacological applications of the carotenoid norbixin

Aplicações farmacológicas do carotenoide norbixina: uma revisão sistemática de literatura

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ABSTRACT

Carotenoids play essential roles in human health and can fight and prevent diseases. One carotenoid that is currently attracting scientific interest, particularly for its antioxidant properties, is norbixin. Studies related to its use in the treatment of diseases have become increasingly present in the medical field. This systematic literature review aimed to investigate and gather studies on the pharmacological activity of norbixin for health benefits and the treatment of diseases. Four databases were consulted: Web of Science, PubMed, Scopus, and ScienceDirect. Articles were extracted using a combination of keywords such as 'norbixin', 'antioxidant', 'diseases', '*in vitro*', '*in vivo*', etc. A total of 173 articles were identified. After applying the inclusion and exclusion criteria, 21 were selected for inclusion in this review. Studies have shown that norbixin, alone or in combination with other treatments, may be effective in DNA protection, tissue engineering, bone repair, and the prevention and treatment of age-related macular degeneration and cardiovascular disease. These results provide a solid basis for further studies aimed at exploring the pharmacological properties of norbixin and enabling its use in therapeutic applications.

Keywords: Carotenoids; Antioxidant properties; Norbixin; Pharmacological activity.

Recebido: 03/04/2024 | Aceito: 05/05/2024 | Publicado: 09/05/2024

RESUMO

Os carotenoides desempenham um importante papel na saúde humana, combatendo e prevenindo doenças. Um carotenoide que atualmente desperta interesse científico, principalmente por suas propriedades antioxidantes, é a norbixina. Esta revisão sistemática da literatura teve como objetivo investigar e reunir estudos sobre a atividade farmacológica da norbixina para benefícios à saúde e tratamento de doenças. Quatro bases de dados foram consultadas: Web of Science, PubMed, Scopus e ScienceDirect. Os artigos foram extraídos utilizando uma combinação de palavras-chave como 'norbixina', 'antioxidante', 'doenças', '*in vitro*', '*in vivo*' etc. Foram identificados 173 artigos. Após aplicação dos critérios de inclusão e exclusão, 21 foram selecionados para inclusão nesta revisão. Estudos demonstraram que a norbixina, isoladamente ou em combinação com outros tratamentos, pode ser eficaz na proteção do DNA, na engenharia de tecidos, na reparação óssea e na prevenção e tratamento da degeneração macular relacionada à idade e de doenças cardiovasculares. Os resultados fornecem uma base sólida para novos estudos que visam explorar as propriedades farmacológicas da norbixina e permitir a sua utilização em aplicações terapêuticas.

Palavras-chave: Carotenoides; Propriedades antioxidantes; Norbixina; Atividade farmacológica.

INTRODUCTION

Annatto (*Bixa orellana* L.), a native plant from Central and South America, is one of the most widely used sources of carotenoids. For example, bixin and norbixin are extracted from the seed of annatto (Roehrs *et al*., 2014). Although mainly used as food colorants, bixin and norbixin have properties that make them interesting for applications in other fields, such as in the health sector. Both are available for use by the industry as natural antioxidants, offering a substitute that can reduce or replace synthetic additives (Garcia *et al*., 2012).

According to Satyanarayana *et al*. (2003), the primary pigment of annatto is bixin, corresponding to more than 80% of the carotenoids found in these seeds. Removal of the methyl ester group from bixin leads to norbixin, a dicarboxylic acid. The structural differences give the bixin fat-soluble characteristics due to the presence of the methyl ester in the molecule. At the same time, norbixin presents higher water solubility due to the presence of the carboxyl group (Lima *et al*., 2001).

The natural substance norbixin is a simple diacid that is biocompatible, does not harm living things, and has antioxidant properties that make it work against free radicals (Beni *et al*., 2020; Sousa *et al*., 2020). This carotenoid has anti-inflammatory, antimicrobial, antitumor, antigenotoxic, and antimutagenic characteristics (Alves *et al*., 2018; Fontaine *et al*., 2021; Nascimento *et al*., 2020; Roehrs *et al*., 2017). Literature says that norbixin's chemical properties make it useful not only as a food color but also in the making of biomaterials like biodegradable and biocompatible polymer membranes (Alves *et al*., 2018; Fontaine *et al*., 2021; Nascimento *et al*., 2019; Sousa *et al*., 2021).

Several experimental studies involving the applications of norbixin in the health sector have been conducted, which in turn makes the already published knowledge in this respect interesting. Systematic Literature Reviews (SLRs) can be useful to summarize and characterize the state of the art of the research area. The review conducted by Saini *et al*. (2022) critically analyzed the chemistry and antioxidant activity of carotenoids and some of their health benefits. Pilai, Soni and Dhulap (2024) conducted an SLR in which they sought to build a scientific survey and patent documents on the pharmacological and cosmeceutical applications of *Bixa orellana*. Unlike these reviews, the SLRs reported here aim to gather, critically evaluate, and synthesize existing research studies on the biological activity of norbixin for health and the benefits and treatment of diseases.

METHODS

This systematic review was conducted following the guidelines described in the Preferred Report Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher *et al*., 2015). The study was registered in PROSPERO (record: CRD42023415027). The production of this SLR followed three steps: planning, conduction, and production of the report (Kitchenham; Charters, 2007). The steps were developed through an online tool for the collaborative conduct of SLRs, Parsif.al.

Research Questions

The research questions (RQs) for this SLR are:

- RQ1: What properties and characteristics of norbixin make this molecule efficient for use in health care?
- RQ2: Norbixin is associated with the treatment and prevention of what types of diseases?
- RQ3: What is the biological performance of norbixin *in vivo* or *in vitro* tests?
- RQ4: What are the possible risks and adverse effects of using norbixin?

Research Strategies

To answer the RQs, the PICOC strategy was used, according to Table 1, which refers to essential components of formulating a research question, where P = participant, I = intervention, C = comparison, O = outcome, and C = context. A systematic search based on the items as described in Table 1 was performed until April 10, 2024.

Table 1 – PICOC strategy.

P	Rats, mice, rabbits, and cells
I	Pharmacological activity of norbixin
C	Efficacy and pharmacological use with medicines and other carotenoids
O	Pharmacological activity; Number of diseases treated; doses used in treatments
C	Experimental research in vitro and in vivo using only norbixin, norbixin in the composition of biomaterials or in joint action with other treatments

Source: Authors (2024).

A search string was elaborated and applied, as shown in Table 2. The terms were chosen to maximize the number of articles on the topic. The search string was applied to the four article databases listed in Table 2. After obtaining the articles, duplicate entries were removed with the support of the Parsif.al tool.

Table 2 – Search string and article databases.

Search string	Article Sources
(Norbixin) AND (Antioxidant OR Disease OR Illness OR "In Vitro" OR "In Vivo")	PubMed, Scopus, Web of Science and Science Direct

Source: Authors (2024).

Selection Criteria

Initially, two researchers were responsible for applying the selection criteria of the articles had read only the titles and abstracts of the studies. A concordance between the researchers was assessed using Cohen's Kappa coefficient (Cohen, 1968). Discrepancies were resolved through meetings. When necessary, a third researcher contributed to the discussion to resolve conflicts. The criteria for selecting articles are specified in Table 3.

Table 3 – Criteria for inclusion and exclusion of articles.

Inclusion criteria	Exclusion criteria
Primary experimental study looking at the pharmacological activity of norbixin	Primary experimental study not directly related to the topic: pharmacological activity of norbixin
Experimental research *in vivo* and/or *in vitro*	Research without *in vivo and*/or *in vitro* studies
Articles written in English	Articles not written in English
Only primary studies	Secondary or tertiary studies
Full articles	Full text not available in databases
Peer-reviewed articles	Gray literature (theses, dissertations, short articles, book chapters, technical reports, etc.)

Source: Authors (2024).

Subsequently, the researchers reviewed independently the full text and included the articles in the study. To ensure that all relevant studies were included, listed scientific reference studies, and cited references were conducted based on articles that met the study selection criteria (backward snowballing technique) (Wohlin, 2014). The researchers

selected the eligible articles identified at this stage and evaluated them using the same selection criteria.

Quality Assessment

The selected articles underwent a quality assessment using the ToxRTool tool, with adaptations made by the authors (NHMRC, 2019). The adapted checklist consisted of sixteen criteria for *in vivo* studies (Group 1), thirteen for *in vitro* studies (Group 2), and eighteen for studies involving both types of experimental research (Group 3), covering different aspects of the risk of bias. The criteria were organized, in both cases, in five groups: identification of the test substance, characterization of the test system, description of the study design, documentation of the study results, and plausibility of the design and study results (Tran *et al*., 2021). The following score was attributed to each quality question, according to the answers: yes = 1 point, partially = 0.5 points, and no = zero. Two independent researchers conducted the quality analysis. In cases where disagreements arose, a third reviewer was consulted. The study selection process is outlined in Figure 1, which presents the preferred report items for systematic reviews.

Data Extraction

Researchers carried out this stage of the study in a standardized manner using a data extraction form. The following information was extracted from each included study: identification of the article (title, authors, year, and country where the research was conducted), type of study (*in vivo* and/or *in vitro*), methodologies used, population tested (cell type or animal species) dose/concentration used and duration of treatment (norbixin and controls), and results. All data were extracted using the Parsif.al tool.

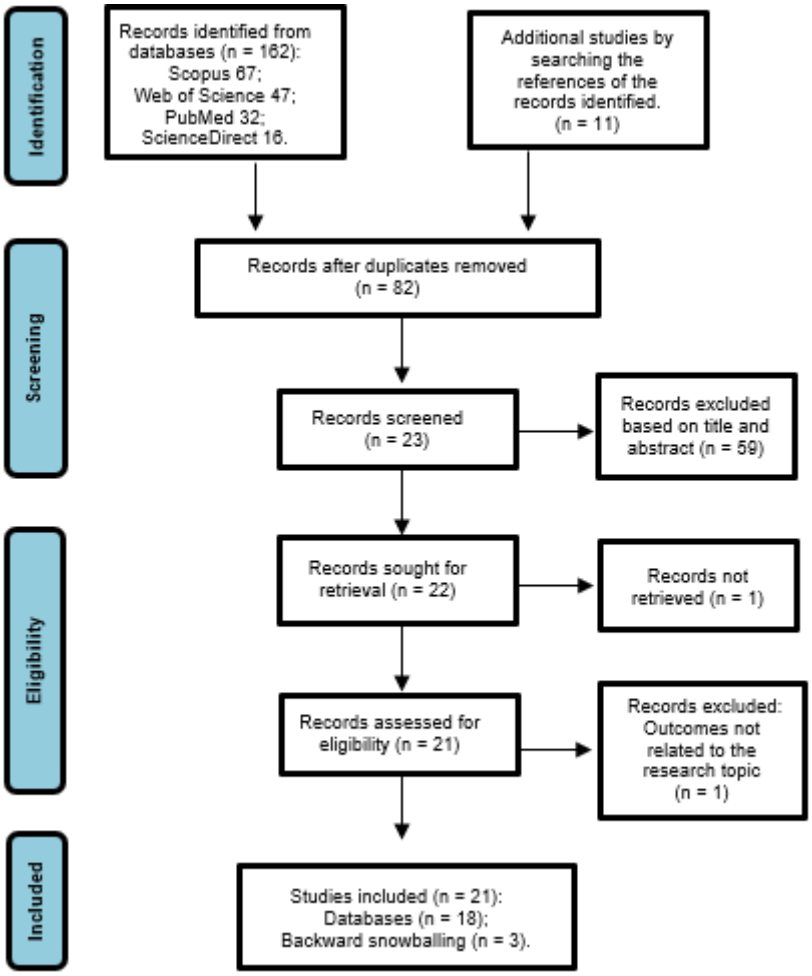
RESULTS

Search and Selection of Eligible Studies

Figure 1 illustrates the selection process in this systematic review. To verify the degree of concordance between the reviewers, after the independent selection performed by each researcher, Cohen's Kappa coefficient was calculated. The Kappa result was 0.936, which represents an almost perfect agreement (Viera; Garrett, 2005). Finally, twenty records were identified directly from the databases as eligible for full-text analysis, and eighteen were selected. By searching the references of these papers (backward snowballing technique), nine more records were obtained for full reading. The records were evaluated by the same researchers involved in the selection phase until reaching a

consensus, and two papers were selected. In the end, a total of twenty articles were included in the review. By searching the references of these articles (backward snowballing technique), nine more records were obtained for full reading. The records were evaluated by the same researchers involved in the selection phase until a consensus was reached, and two works were selected. In the end, a total of twenty articles were included in the review.

Figure 1 – PRISMA flow diagram.

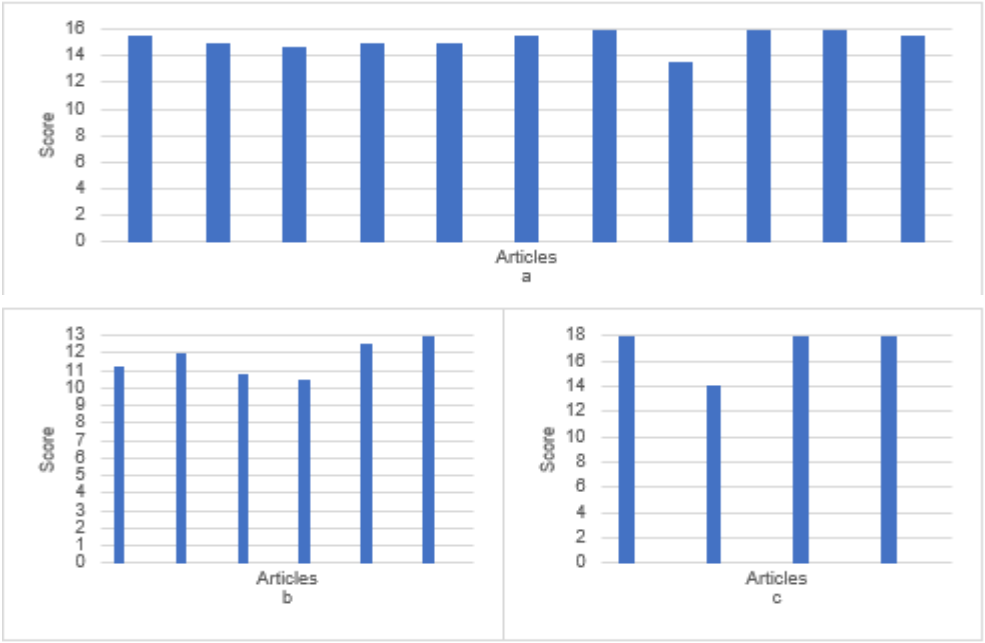


Source: Adapted from PRISMA (Page et al., 2021).

Quality of the selected studies

Figure 2 shows the result of the quality assessment of the selected articles, organized into groups: 1 (*in vivo*), 2 (*in vitro*), and 3 (*in vivo* and *in vitro*).

Figure 2 – Scores of studies in group 1 (a), group 2 (b), and group 3 (c), obtained in the quality assessment.



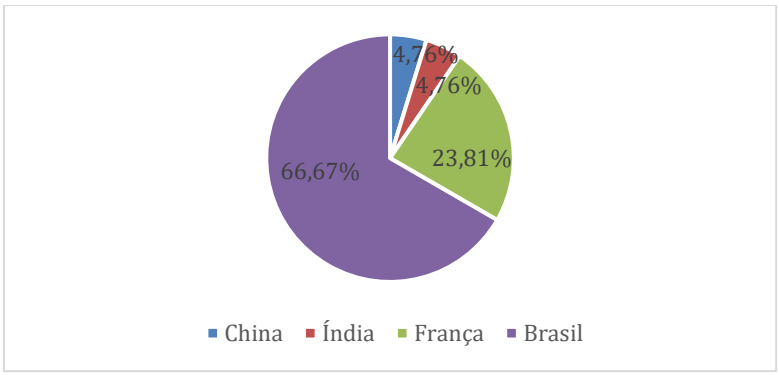
Source: Authors (2024).

The studies were evaluated, and seven articles received the maximum points, with three from Group 1, one from Group 2, and three from Group 3. The minimum score in each group was 13.5 for an article in Group 1 (84.4%), 10.5 for an article in Group 2 (75% of the score), and 14 for an article in Group 3 (84.4%). Overall, the studies provided reliable results and met their objectives.

Characteristics of the selected studies

The articles included are research conducted in the following countries: China, India, France, and Brazil; published between 2001 and 2024, as shown in Figures 3 and 4.

Figure 3 – Countries of origin of the papers.



Source: Authors (2024).

Figure 4 – Number of articles published per year.

Source: Authors (2024).

The characteristics and main results of the twenty-one articles selected in this SLR are described in Tables 4 (in vivo studies), 5 (in vitro studies), and 6 (in vivo and in vitro studies). The papers are organized in chronological order of publication. The pharmacological effects of norbixin reported in the studies offer good prospects for the future use of the carotenoid in clinical practice.

Table 4 - Characterization of *in vivo* studies.

Reference	Objectives	Experimental analyses	Population / Sample	Treatment / Duration	Results
Santos et al. (2002)	To investigate the effects of NBIX on plasma lipid metabolism and plasma arylesterase/paroxonase activity, and to evaluate its efficacy in preventing the formation of hepatic lipid peroxides under conditions of high fat consumption.	Biochemical analytical determinations, Plasma arylesterase/ paraoxonase activity assay, Basal and induced lipid peroxidation levels, Determination of thiobarbituric acid-reactive substances.	Male Swiss mice.	2 doses of norbixin: 7.7 mg/kg (for both diets) and 77.7 mg/kg (control diet) or 92.8 mg/kg (high-fat diet); 4 weeks.	NBIX alters plasma lipid levels and paraoxonase activities in Swiss mice fed an atherogenic diet.
Barcelos et al. (2012)	To investigate the potential protective effects of BIX and NBIX against the side effects of MeHg.	Comet assays with peripheral leukocytes and hepatocytes, Catalase activity, Reduced-Glutathione levels, Measurement of mercury in liver and blood.	Male Wistar rats	MeHg (30 µg/kg/ bw/day), BIX or NBIX (0.1-10 mg/kg/bw/day), combinations of the metal compound and the carotenoids, for 45 days.	Consumption of BIX and NBIX may protect humans against adverse health effects caused by mercury exposure.
Roehrs et al. (2014)	To investigate whether BIX and NBIX can prevent hyperglycemia, dyslipidemia and oxidative stress associated with diabetic rats, induced by streptozotocin.	Biochemical Analyses, Biomarkers of Oxidative Stress.	Male Wistar rats	10 and 100 mg/kg of BIX or NBIX; up to 2 mL/kg of body weight, daily administration for 30 days.	Unlike BIX, NBIX did not protect against hyperglycemia and dyslipidemia, and at the highest dose increased dyslipidemia and oxidative stress in the streptozotocin diabetes model.

Rovani et al. (2016)	To evaluate the effects of NBIX in a model of gastric ulcer induced by EtOH in rats.	Macroscopic and histopathological analysis, quantification of NPSH, LPO measurement, CAT and protein quantification.	Male Wistar rats	NBIX (10% m/v) at doses of 0, 10 and 25 mg/kg of body weight; Single dose.	NBIX did not protect gastric tissue against EtOH damage, increased LPO in the gastric mucosa, caused CAT inhibition and NPSH depletion.
Rohini et al. (2018)	To evaluate the effect of NBIX in the amelioration of CMetS by computational and experimental methods.	Biochemical estimation in serum and heart, Haemodynamic assessment, Western blot analysis, Histopathological studies.	Male Wistar albino rats	High-fat diet and NBIX in water, in low and high doses ranging from 3 to 10 mg/kg and 30 to 60 mg/kg, for 60 days.	The results validate NBIX as a new PPARγ agonist and demonstrate the therapeutic potential of NBIX in the treatment of CMetS.
Alves et al. (2018)	To assess the therapeutic effect of 780-nm laser PBM and PSNC on rats with calvarial bone defects.	Surgical procedure, Laser PBM protocol, Histological analysis, SEM analysis of bone and membrane, Confocal Raman spectroscopy.	Male Wistar rats.	Bone defect filling with PSNC membrane and PBM laser irradiation on alternate days for 15 and 30 days.	The membrane coated with NBIX reduced the inflammatory process and served as a scaffold for bone repair when used in isolation.
Nascimento et al. (2019)	To evaluate the effect of NBIX-based PHB membranes on the tendon repair process after tenotomy in terms of the progression of the inflammatory process and the induction of type I and III collagen production.	Surgical procedure; Histological analysis.	Male Wistar rats.	NBIX-based PHB membrane in post-tenotomy tendon repair; treatment for 7, 14 and 21 days.	The membrane promoted the tissue repair process by reducing the inflammatory response, stimulating fibroblast proliferation and collagen remodeling.
Fontaine et al. (2020)	To evaluate the effectiveness of systemic administrations of NBIX during BLD in mice.	Intraperitoneal treatment and BLD; Kinetics of ERGs, photoreceptor loss and A2E accumulation in mice; Full-field electroretinogram; Histology and photoreceptor counting; Electronic microscopy analysis; A2E measurement by HPLC-MS/MS; A2E measurement in mice eyes; NBIX concentration determination in pellets, mice plasma and eye samples.	BALB/c mice and Abca4$^{-/-}$ Rdh8$^{-/-}$ mice	NBIX (10 mg/kg in 5 % Tween 80 in PBS) 30 min prior to light damage and 1, 2.5 and 4 hours after the beginning of the blue light exposure. Daily norbixin dose of 47.5 mg +/- 5 mg per kg for up to 6 months.	Administration of NBIX in the BLD model of dry AMD is neuroprotective and partially preserves photoreceptor function. Chronic supplementation with NBIX reduces ocular levels of A2E, is neuroprotective, and preserves visual function, modeling retinal degenerative diseases such as DMGT and dry AMD.
Nascimento et al. (2020)	To evaluate the in vivo response of photobiomodulation therapy associated with NBIX-based PHB membrane in tenotomized calcaneal tendon.	Surgical procedure, Experimental protocol, Euthanasia, Histological technique.	Male Wistar rats.	Low-intensity photobiomodulation associated with NBIX-based PHB membrane; daily LED irradiation until euthanasia (7, 14, and 21 days).	Photobiomodulation in conjunction with the membrane led to control of the inflammatory process, but did not favor fibroblast proliferation, nor did it optimize the formation of type I collagen.

Sousa et al. (2020)	To synthesize and characterize PHB and norbixin membranes to evaluate them for genotoxicity in rats and wound healing in mice by histological staining.	Genotoxicity assessment; Wound healing assessment (Induction of experimental injury and Histological analysis).	Rattus novergicus male	5% PHB/ NBIX membrane; 72 h for genotoxicity assessment; 7 and 14 days for wound healing assessment.	The membrane reduced the inflammatory process and served as a scaffold for wound healing due to the stimulus to reepithelialization.
Sousa et al. (2021)	To synthesize and verify the effectiveness of the polyhydroxybutyrate and NBIX membrane as a scaffold in bone defects induced in the tibia of rats.	Induction of bone defects, Analysis by Raman spectroscopy, Scanning electron microscopy analysis, Histopathological analysis.	Male rats (Rattus norvegicus) .	Implantation of the membrane based on PHB and norbixin at the site of the lesion, treatment for 15 and 30 days.	The membrane acted as a support in the repair of bone defects. After 15 days, there was a significant increase in the deposition of organic and inorganic matrix.

Abbreviations: NBIX, norbixin; BIX, bixin; MeHg, methylmercury; EtOH, ethanol; NPSH, non-protein sulfhydryl groups; LPO, lipid peroxidation; CAT, catalase activity; CMetS, cardio-metabolic syndrome; PPARγ, peroxisome proliferator-activated receptor γ; PBM, photobiomodulation; PSNC, polystyrene membranes coated with norbixin and collagen; SEM, Scanning Electron Microscopy; PHB, poly(hydroxybutyrate); BLD, blue light damage; ERGs, electroretinogram; AMD, Atrophic age-related macular degeneration; STGD, Stargardt disease; A2E, N-retinylidene-N-retinylethanolamine; LED, Light Emitting Diode.

Source: Authors (2024).

Table 5 - Characterization of *in vitro* studies.

Reference	Objectives	Experimental analyses	Population / Sample	Treatment / Duration	Results
Kovary et al. (2001)	To evaluate the biochemical behavior of NBIX under conditions of DNA damage induced by different generation systems of reactive oxygen species.	Oxidative damage of plasmid DNA by stannous or ferrous iron ions, Transformation efficiency of Escherichia coli cells by plasmid DNA, Antimutagenicity assay, Mammalian cell culture and cytotoxic assays, Single-cell gel electrophoresis, Quantitation of DNA lesions.	Plasmid DNA, Escherichia coli cells, Salmonella typhimurium strain TA102, Balb/c 3T3 fibroblasts.	Different amounts of NBIX in water and at different concentrations (up to 450 mM) for periods of 24, 72 and 96 h.	NBIX has the potential to protect DNA against oxidative damage induced by H_2O_2 and metal ions.
Júnior et al. (2005)	To evaluate the effect of NBIX on the response of Escherichia coli cells to DNA damage induced by UV radiation, H_2O_2 and superoxide anion.	Isolation of NBIX, Survival experiments, beta-galactosidase chromotest and antimutagenicity assay.	Escherichia coli cells.	2 mM NBIX; some hours.	NBIX protects Escherichia coli cells against DNA damage induced by UV radiation, H_2O_2 and O_2^{2-}, in addition to having antimutagenic properties in tests with Salmonella.
Ouyang et al. (2008)	To explore the biochemical behavior of annatto and its derivatives by evaluating the effects of NBIX on copper (II) ion-mediated DNA damage in supercoiled plasmid DNA.	Plasmid DNA cleavage.	pBR322 plasmid DNA prepared from DH5α cells.	NBIX (1 to 1000 μM) without and with 50 μM Cu^{2+}; some hours.	The addition of NBIX, in a concentration and interaction time-dependent manner, increased the DNA damage induced by copper (II) ions.

Beni et al. (2020)	To investigate the in vitro effect of BIX and NBIX on erythrocyte membrane resistance to hemolysis and ex vivo osmotic fragility of human erythrocytes after a 7-day dietary supplementation with placebo, BIX or NBIX.	Assessment of erythrocyte osmotic fragility and Index of erythrocyte hemolysis and stability.	Human erythrocytes.	BIX (0.3-10 μmol/L), NBIX (0.03-10 μmol/L); some hours.	Supplementation with BIX and NBIX increased erythrocyte membrane resistance. BIX and NBIX protected erythrocytes from lipid peroxidation, improved the cellular redox environment, reduced erythrocyte membrane fragility induced by AAPH, glucose, or NaNO2, and improved basal osmotic resistance.
Fontaine et al. (2021)	To test the effects of A2E in the presence or absence of norbixin on the transactivation of PPAR and RXR and on the expression of inflammatory molecules and VEGF in porcine primary RPE cells in vitro.	Binding studies to RXR-α and PPAR-α and γ; In vitro model of RPE cell culture and treatments; PPAR, RXR, AP-1 and NF-κB transactivation assays; RT-PCR; Protein analysis.	RPE cells.	NBIX (20 μM); some hours.	NBIX partially inhibits RXR transactivation, acts as a pan-inhibitor of A2E-induced PPAR transactivation, and modulates the expression of molecules involved in A2E-stimulated angiogenesis and inflammation.
Fontaine et al. (2024)	To examine the roles of RAR, PPAR and RXR transactivation in cell death, angiogenesis and A2E-induced inflammation in an in vitro model of AMD, using various RAR antagonists.	NBIX and A2E synthesis; EPR phototoxicity and treatments; RAR-α binding studies; protein analysis; quantitative RT-PCR; RAR, PPAR, RXR, AP-1 and NF-κB transactivation assays.	RPE cells; Human recombinant RARα expressed in insect cells.	NBIX (20 μM); BMS 195614 (10 μM); AGN 193109 and BMS 493 (minimum dose of 0.1 μM); some hours.	RAR inhibitors and NBIX demonstrated photoprotective and anti-inflammatory effects in A2E-stimulated RPE cells *in vitro* by non-specifically modulating PPAR or RXR transactivation.

Abbreviations: DNA, deoxyribonucleic acid; H_2O_2, hydrogen peroxide; UV, ; O_2^{2-}, superoxide anion; AAPH, 2,2'-azobis(2-amidinopropane) dihydrochloride; $NaNO_2$, sodium nitrite; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; VEGF, vascular endothelial growth factor; RPE, retinal pigment epithelium; AP-1, activator protein 1; NF-κB, nuclear factor kappa B; RT-PCR, real-time polymerase chain reaction; RAR, retinoic acid receptor.

Source: Authors (2024).

Table 6 - Characterization of *in vivo* and *in vitro* studies.

Reference	Objectives	Experimental analyses	Population / Sample	Treatment/ Duration	Results
Fontaine et al. (2016)	To evaluate the efficiency of BIX and NBIX in specific in vitro tests with primary cultures of porcine RPE and a set of in vivo experiments.	In vitro model of phototoxicity and RPE treatments; BLD in vivo, histology and photoreceptor Count; Measurement of A2E in RPE cells or culture medium; A2E measurement in the eyes; Analysis of oxidized forms by MS; Pharmacokinetic studies of bixin and norbixin in mice; Analysis of mice plasma and eye samples for bixin content.	RPE cells (in vitro); Abca4$^{-/-}$ Rdh8$^{-/-}$ mice and male Sprague-Dawley rats (in vivo).	*In vitro*: BIX, NBIX and other carotenoids in DMSO (5, 10, 20 and 50 μM), duration of a few days. *In vivo*: 2 μL of 500 μM NBIX in DMSO (intravitreal injection in one eye), intraperitoneal injection with norbixin (10, 50, 100 mg/kg), oral BIX or NBIX (50 mg/kg, in DMSO and Isio4 oil) or intraperitoneally (5 mg/kg, in DMSO/ Tetraglycol/ H_2O); duration of 3 months: 2.5 mg norbixin/day, dissolved in DMSO and Tween 80, orally in drinking water.	BiX and NBIX protect RPE cells against A2E-induced phototoxicity in vitro and are more efficient than the other carotenoids. NBIX reduces A2E accumulation by RPE cells in vitro and mice RPE in vivo. NBIX protects the retina of rats and mice against blue light-induced phototoxicity.

Somacal et al. (2022)	To investigate the ability of NBIX to protect against LDL oxidation *in vitro* and its antioxidant and anti-inflammatory properties in rabbits fed with an atherogenic diet.	In vitro assessment of LDL oxidation (Human LDL isolation and LDL oxidation assays); Oxygen radical absorbance capacity assay; In vivo study in a rabbit model of atherosclerosis (Serum biochemical measurements, Serum norbixin levels, Histopathological analysis and Assessment of oxidative and antioxidant markers in aortic tissue).	Human LDL; Male New Zealand rabbits.	*In vitro*: different concentrations of norbixin (0 – 7.5 µM). *In vivo*: atherogenic diet (0.5% cholesterol) alone or supplemented with norbixin (10, 30 or 100 mg/kg of body weight) for 60 days.	NBIX inhibits oxidation of human LDL; increases HDL levels and reduces levels of oxidized LDL, autoantibodies, and triglycerides; inhibits oxidation of aortic tissue lipids and proteins; and neutralizes alterations in endogenous antioxidant defense systems in atherogenic rabbits. Dietary NBIX may be atheroprotective and reduce risk factors for cardiovascular disease.
Fontaine et al. (2023)	To examine the mode of action and the in vitro and in vivo effects of BIO203, a novel NBIX amide conjugate.	Stability of NBIX and BIO203 at different temperatures; EPR phototoxicity and cytokine expression; PPAR, RXR, AP-1 and NF-κB transactivation; Quantitative RT-PCR; BLD study and acute ocular pharmacokinetics studies in rats; Electroretinogram, histology and thickness measurement of the ONL of the retina in rats and mice; Oral treatment by dietary supplementation; determination of NBIX and BIO203 in plasma and of A2E in mice eyes.	RPE cells; Male Sprague-Dawley rats, Abca $4^{-/-}$ Rdh $8^{-/-}$ mice.	*In vitro*: NBIX or BIO23 (1, 2 ,5 10, 15 and 20 µM); *In vivo*: doses of 5, 10 and 25 mg/kg of BIO203 injected intraperitoneally in 4 moments: 30 min before BLD and 1, 2.5 and 4 h after the start of exposure to blue light; NBIX (10 mg/kg) and BIO203 (8.8 mg/kg) dissolved in vehicle, single dose; NBIX (10 mg/kg) and BIO203 (2.5 mg/kg) dissolved in vehicle, 4 successive doses; 46 µg/g ± 3.5 µg/g and 464 µg/g ± 24.3 µg/g of BIO203 in feed pellets, orally for 6 months.	BIO203 was photoprotective in vitro, inhibited the transactivation of NF-κB, AP-1 and the expression of VEGF, IL-8 and IL-6, which are involved in angiogenesis and inflammation critical for AMD. In vivo, BIO203 and NBIX were neuroprotective and limited the loss of visual function in two models of retinal degeneration.
Franklin et al. (2023)	To determine the antimicrobial and antioxidant activity of the aqueous extract of annatto seeds and its healing potential in exposed skin lesions.	Extraction and estimation of bixin and norbixin, Antioxidants assay, Antimicrobial assay, wound skin healing.	Male Wistar rats; Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922).	*In vitro*: 4 mL of norbixin solution with 150, 75, 37.5 and 18.75 mg, with the addition of 1 mL of inoculum (15 × 108 CFU/mL) in 3 tubes. *In vivo*: 1 mL of 10% aqueous extract (norbixin) in gel, Daily application to wound and evaluation on days 0, 7 and 14.	The gel with aqueous extract is effective in skin healing in rats, being used as a phytotherapic, besides possessing antioxidant and antimicrobial activity.

Abbreviations: MS, *Mass spectrometry*; DMSO, dimethyl sulfoxide; LDL, low density lipoprotein; HDL, high density lipoprotein; BIO203, novel NBIX amide conjugate; IP, intraperitoneal; ONL, Outer Nuclear Layer; IL-6, interleukin 6; IL-8, interleukin 8.

Source: Authors (2024).

DISCUSSION

The results showed that norbixin has properties that make its application in the medical field interesting. The following topics answer the four research questions that guided the review and are organized according to the type of disease treated by the use of

norbixin, in response to RQ2. For each type of treatment, the properties of the carotenoid studied are reported, as questioned in RQ1, its performance in the experimental tests, according to RQ3, and the adverse effects associated with its use, if mentioned in the studies, in response to RQ4.

The pharmacological activity of norbixin in tissue engineering

In response to question RQ1, in tissue engineering, the main properties exploited by the carotenoid are related to its antioxidant power, biocompatibility, antimicrobial activity and its ease of use in the composition of other products such as gels and polymeric membranes (Trombino *et al*., 2023; Woźniak-Budych, 2021). The studies identified investigated norbixin's properties in protective and healing coatings for wounds and in tissue repair and bone regeneration, as asked for in RQ2. Two membranes and a gel containing annatto seed extract were synthesized using the carotenoid. In terms of its performance in the experimental tests, question RQ3, norbixin showed an anti-inflammatory effect, healing activity and promoted tissue repair.

Nascimento *et al*. (2019) demonstrated that a poly(hydroxybutyrate) (PHB) membrane based on norbixin promoted the tissue repair process in the Achilles tendon of rats by reducing the inflammatory response, increasing fibroblast proliferation, and improving collagen production. A more recent study by the same group tested the norbixin-based PHB membrane in conjunction with photobiomodulation therapy. Although it led to control of the inflammatory process, the combined use of the two treatments showed little efficiency compared to the use of LED therapy alone, as it did not favor fibroblast proliferation and did not optimize the formation of type I collagen in the repair process (Nascimento *et al*., 2020).

In another study, the PHB/norbixin membrane showed promising results as a healing agent. In vivo tests confirmed the absence of toxicity of the biomaterial and its efficacy in reducing the inflammatory process and acting as a scaffold by stimulating re-epithelialization, with complete healing of the induced lesion at the end of treatment (Sousa *et al*., 2020). Franklin *et al*. (2023) in a study developing a gel containing norbixin and 10% aqueous extract of annatto seeds, reported the product's effective action in healing the skin of animals, as well as its antioxidant and antimicrobial activity.

With regard to the use of biomaterials in bone repair, PHB and norbixin membrane had a significant influence on obtaining a high level of mineralization and crystallinity, formation of collagen I matrix, reduction of inflammatory process and superior quality of

newly formed bone tissue in bone defects induced in the tibia of rats (Sousa et al., 2021). A polystyrene membrane coated with norbixin, and collagen (PSNC) effectively reduced the inflammatory process and served as a support for bone repair. Laser photobiomodulation (PBM) was combined with the use of the PSNC membrane, and although PBM showed some positive effects, such as increased deposition and organization of newly formed bone, it failed to improve the bioactive properties of the membrane (Alves *et al*., 2018).

DNA protection

The main property of norbixin related to DNA protection is its action as an antioxidant, as stated in RQ1. Four studies evaluated the effect of norbixin on DNA protection. In conjunction with its antioxidant power, due to the conjugated double bonds present in its structure, norbixin may promote the quenching of free radicals and thus help fight diseases such as cancer, for example, in resolution of RQ2 (Júnior *et al*., 2005; Kovary *et al*., 2021). For RQ3 and RQ4, experimental tests confirmed the effectiveness of the carotenoid's protective action in some cases, but warned of adverse effects such as DNA damage caused by exposure to certain substances or under certain specific conditions (Kovary *et al*., 2001; Ouyang *et al*., 2008).

Kovary *et al*. (2001) showed that norbixin has the potential to protect against oxidative damage to DNA induced by H_2O_2 and metal ions. However, under non-physiological conditions, the carotenoid may increase the extent of oxidative damage. According to Barcelos et al. (2012), administration of norbixin with methylmercury also results in protection against metal-induced DNA damage. Júnior *et al*. (2005) reported that norbixin protects the DNA of *Escherichia coli* cells against UV radiation, H_2O_2 and superoxide anions (O_2^{2-}). Ouyang *et al*. (2008) observed that norbixin increases copper (II) ion-induced DNA damage in a concentration- and time-dependent manner.

Eye disease treatment

The main properties of norbixin that are of interest in ophthalmic research, in response to RQ1, are its anti-inflammatory, photo- and neuroprotective properties. With respect to RQ2, the studies found focus on the treatment of age-related macular degeneration (AMD) and Stargardt's disease (STGD). The experimental results discussed in RQ3 suggest that norbixin may have a neuroprotective function. Additionally, they indicate that it may be effective in preserving the visual function of photoreceptors and inhibiting the accumulation of N-retinylidene-N-retinylethanolamine (A2E) and

lipofuscin in in vitro and in vivo models of age-related macular degeneration (AMD) and Stargardt disease (STGD).

Four studies by the same group of authors analyzed the ocular pharmacokinetic properties of norbixin. In the initial study, it was found that bixin and norbixin had a protective effect on retinal pigment epithelial (RPE) cells against A2E-induced phototoxicity, and reduced A2E accumulation. This finding is significant because A2E accumulation is one of the primary causes of visual impairment in the elderly. Norbixin protected photoreceptors from blue light damage in a mouse model of dry AMD and was as effective as phenyl-N-tert-butylnitrone, a free radical scavenger. The study also compared the high protective effect of bixin and norbixin with that of three other carotenoids: lutein, zeaxanthin and crocetin, which showed no protection (Fontaine *et al*., 2016). In another study, chronic oral administration of norbixin in animal models of AMD and STGD resulted in neuroprotection, preservation of photoreceptor function, reduction of accumulation and inhibition of the deleterious effects of ocular A2E through regulation of peroxisome proliferator-activated receptors (PPARs) and retinoid X receptors (Fontaine *et al*., 2020).

A new amide conjugated to norbixin, BIO203, was evaluated for its mode of action and compared to the carotenoid. BIO203 showed better ocular pharmacokinetic properties and stability than norbixin. Both compounds share a similar mechanism of action involving inhibition of PPARs, reduced transactivation of nuclear factor kappa B and activator protein (AP-1), and reduced expression of A2E-induced interleukins (IL-6 and IL-8) and vascular endothelial growth factor, processes directly involved in inflammation and angiogenesis critical for AMD. According to the authors, the new biomaterial and norbixin could be candidates for effective drugs for the treatment of AMD (Fontaine *et al*., 2023). In the latest work, it was demonstrated by the authors that photoprotection and the anti-angiogenic and anti-inflammatory effects of norbixin are not related to the inhibition of RAR transactivation, but rather with the inhibition of PPAR and RXR transactivation (Fontaine *et al*., 2024).

Cardiovascular disease treatment

With respect to RQ1, the antioxidant and anti-inflammatory properties of norbixin have been of primary interest in studies related to the treatment of cardiovascular disease. Regarding RQ2, the carotenoid has shown atheroprotective potential, being beneficial in the control of risk factors for atherosclerosis, the main cause of the most common types

of cardiovascular disease, as well as in the prevention and treatment of cardiometabolic syndrome (CMetS) (Jebari-Benslaiman *et al*., 2022; Santos *et al*., 2002; Somacal *et al*., 2022). Experimental results have shown that the carotenoid has the potential to act as a PPAR-γ agonist (Goto *et al*., 2010; Rohini *et al*., 2018; Wang *et al*., 2014), a substance of interest for the treatment of CMetS, inhibits the oxidation of human low-density lipoprotein (LDL) in vitro and improves the serum lipid profile in rabbits, increasing the levels of high-density lipoprotein and reducing the levels of triglycerides, autoantibodies, oxidized LDL, atherogenic index, as well as inhibiting lipid and protein oxidation in the aortic tissue of the animals (Somacal *et al*., 2022), as questioned in RQ3. Another study showed that norbixin inhibits lipid peroxidation, alters plasma lipid levels and paraoxonase/alisterase activities in in vivo tests under conditions of high fat intake, but as noted in RQ4, the doses tested were high and would not be suitable for humans (Santos *et al*., 2002).

Other pharmacological activities and treatments

Beni *et al*. (2020), Roehrs *et al*. (2014), and Rovani *et al*. (2016), investigated the antioxidant power of norbixin, in response to question RQ1, for the prevention of diseases associated with erythrocyte hemolysis, the treatment of gastric ulcers, and the prevention of hyperglycemia, dyslipidemia and oxidative stress, in response to question RQ2. Regarding RQ3, the first study cited reported the efficacy of bixin and norbixin in increasing the resistance of human erythrocytes to hemolysis and preventing oxidative damage. Both carotenoids protected cells against fragility induced by 2,2'-azobis(2-amidinopropane) dihydrochloride, glucose, and sodium nitrite ($NaNO_2$) in *in vitro* experiments and protected erythrocytes from lipid peroxidation by improving the cellular redox environment (Beni *et al*., 2020). The other two studies showed adverse effects in their experimental results in response to RQ4. While bixin was found to be effective in protecting against streptozotocin-induced hyperglycemia and dyslipidemia in diabetic rats, norbixin did not show a protective effect and increased dyslipidemia and oxidative stress at the highest dose tested (Roehrs *et al*., 2014). Norbixin did not protect gastric tissue from ethanol toxicity in a rat model of peptic ulcer. Pro-oxidant effects were observed with treatment, with an increase in gastric mucosal lipid peroxidation, inhibition of catalase activity, and depletion of non-protein sulfhydryl groups (Rovani *et al*., 2016).

Limitations

Some limitations related to the conduction of this SLR should be acknowledged. This SLR was limited to four article databases. Only articles written in English were included, and gray literature articles that may have yielded interesting results were not considered.

CONCLUSION

The systematic literature review developed on the pharmacological activity of norbixin for health benefits and treatment of diseases demonstrated that carotenoid has a promising potential for use in the medicinal field. A total of twenty-one published articles were analyzed, with their data extracted to answer the research questions. The evaluated studies reported different applications and results, through in vivo and in vitro experimental tests. Consistent evidence from studies confirms that norbixin acts as an effective antioxidant agent, which is its main characteristic explored in research. The articles analyzed showed that norbixin is a versatile natural product with effective results in the prevention and treatment of various diseases in animals, either alone or in the composition of extracts, gels, polymeric membranes, in combination or not with other treatments. While norbixin has the potential to be a valuable therapeutic option for humans, further studies are needed to support and solidify its safe use in clinical practice.

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