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The use of oxidative stress biomarkers in the study of exposure to pesticides - a review

O uso de biomarcadores de estrese oxidativo no estudo de exposição á agrotóxicos - uma revisão

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Abstract

The extensive use of chemical compounds in agriculture has been related to the occupational exposure of farmers, in addition to environmental degradation and contamination. Such factors can potentiate the imbalance between oxidant and antioxidant compounds, thus allowing the excessive production of reactive oxygen species, which can damage biomolecules and lead to various diseases, including Alzheimer's and cancer. To correlate the toxic effects with exposure to pesticides, oxidative stress biomarkers have become a viable alternative, as they are more stable, specific, and sensitive molecules, with greater measurement capacity, being able to express normal and pathological biological processes or pharmacological responses to therapeutic interventions. Thus, this work aimed to evaluate the application of the use of oxidative stress biomarkers in the study of exposure to pesticides. After reviewing the literature in online databases (PubMed, Science Direct, Web of Science, and Google Scholar), it was possible to conclude that different parameters have been used as fundamental tools during all stages of risk assessment after exposure to pesticides.

Keywords: Occupational health; Pesticides; Oxidative stress; Biomarkers; Biomonitoring.

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Resumo

O uso extensivo de compostos químicos na agricultura tem sido relacionado com a exposição ocupacional de agricultores, além da degradação e contaminação ambiental. Tais fatores, podem potencializar o desequilíbrio entre os compostos oxidantes e antioxidantes, permitindo, assim, a produção excessiva de espécies reativas de oxigênio, o que pode causar danos às biomoléculas e originar diversas doenças, dentre elas o Alzheimer e o câncer. Para correlacionar os efeitos tóxicos à exposição aos agrotóxicos os biomarcadores de estresse oxidativo se tornaram alternativa viável, pois são moléculas mais estáveis, específicas e sensíveis, com maior capacidade de mensuração, podendo expressar processos biológicos normais e patológicos, ou respostas farmacológicas a intervenções terapêuticas. Assim, este trabalho teve como objetivo avaliar a aplicação do uso de biomarcadores de estresse oxidativo no estudo da exposição aos agrotóxicos. Após a revisão da literatura em bases de dados online (PubMed, Science Direct, Web of Science e Google Scholar), foi possível concluir que diferentes parâmetros têm sido usados como ferramentas fundamentais durante todas as etapas da avaliação de risco após a exposição a agrotóxicos.

Palavras-chave: Saúde Ocupacional; Agrotóxicos; Estrese oxidativo; Biomarcadores; Biomonitoramento.

INTRODUCTION

The use of chemical substances in agriculture to achieve large-scale food production began in Europe at the end of World War II. During the war, pesticides were synthesized as compounds intended for war purposes, but the end of the conflicts transformed the chemical industry into an industry of agricultural pesticides and fertilizers (Mcleish, Johnstone, Schot, 2022; Raffa, Chiampo, 2021). In this way, the end of the War proposed a new field of application for pesticides, now in agriculture, due to its biocidal action and increase in food production, since post-war Europe was a continent of food poverty due to food shortages. resulting from the destruction of fields and plantations (Thomine *et al*., 2022). However, the indiscriminate use of these substances has had negative consequences for the environment and the health of the population, especially for those who have direct contact with pesticides, causing the development and worsening of diseases such as cancer, diabetes, neurological and reproductive disorders, and teratogenicity (Sabarwal, Kumar, Singh, 2018; Tarboush *et al*, 2022).

Due to the high and still underestimated toxicity of these compounds and the frequent increase in concentrations in different environmental compartments, exposure to these agents has become a public health problem (Carvalho, 2017; Dhananjayan, Ravichandran, 2018).

As a result of popularization and inappropriate use, a large part of the population is directly or indirectly exposed to these chemical agents (Dhananjayan, Ravichandran, 2018). Direct exposure occurs, above all, by workers involved in the manufacture, formulation, and application of pesticides. However, the general population can also be exposed to pesticide residues present in food, water, domestic use, or residence near agricultural fields since these are easily dispersed by the action of the wind (Dhananjayan, Jayakumar, Ravichandran, 2020; Rousis, Uccato, Castiglioni, 2016; Townsend *et al*., 2017). However, even at lower concentrations, exposure can cause adverse effects and represent a high risk for exposed living beings, especially humans (Machado, Martins, 2018).

To assess the toxic effect related to the use of pesticides, biomarkers can be used, as most of these substances are rapidly metabolized and do not accumulate in the body (Machado, Martins, 2018). Biomarkers are important tools in epidemiological studies, as they allow for establishing the relationship between exposure to chemical agents and their effects on exposed individuals (Cattelan *et al*., 2018). Thus, the determination of biomarkers accurately represents the exposure situation and allows the monitoring of the health of individuals as well as of the ecosystem in general, in addition to the early detection of the action of these xenobiotics (Torres *et al*., 2018). However, the validation of biological indicators requires care in relation to specificity and sensitivity, and they can be used for different purposes, depending on the purpose of the study (Machado, Martins, 2018). Thus, oxidative stress biomarkers have proven to be efficient for biological and environmental monitoring purposes, since they make it possible to analyze changes in the genetic material of organisms with high sensitivity and low cost (Beedanagari, Vulimiri, Mahadevan, 2014; Nagy *et al*., 2019).

The onset of oxidative stress in the metabolic system results from the existing imbalance between oxidant and antioxidant compounds due to the incessant production of free radicals or to the detriment of their removal speed from the metabolic system. This imbalance is caused by several factors, such as exposure to chemical, physical and biological agents (Liguori *et al*., 2018; Sabarwal, Kumar, Singh, 2018). Physiologically, this imbalance causes damage to biomolecules, which partially or totally lose their biological activities, promoting an imbalance in the organism, which is expressed through cellular, tissue, and organ damage, which can trigger a series of pathological events in the organism (Sies, Jones, 2020).

Human assessment, through effect and exposure indicators, can correlate the data with the environmental assessment, allowing one to infer whether a given symptom, complaint, or disease may be related to exposure to pesticides. It also allows for establishing a limit considered acceptable by regulatory agencies for exposure to the studied chemical agents (Dalmolin *et al*., 2020).

Thus, to assess the toxic effects associated with the use of pesticides, oxidative stress biomarkers have proven to be an alternative tool for quantitative analysis and may serve as an instrument to relate the genesis of diseases in individuals, enabling the control and prevention of various diseases of effectively for populations that are under the same exposure to pesticides (Raffa, Chiampo, 2021; Lozano-Paniagua *et al*., 2018). Thus, this study presents a review of the use of oxidative stress biomarkers as a toxicological analysis tool to assess the risk of exposure to this class of substances.

METHODOLOGY

This review study was based on a non-systematic methodological approach. Thus, for articles, standardized searches were carried out in different online electronic databases (PubMed, Science Direct, Web of Science, CAPES, and Google Scholar) from 2016 to 2022, that is, the last 5 years, using specific descriptors, with the aim of compiling information on the use of oxidative stress biomarkers used to identify exposure to pesticides. The main descriptors were “Agrochemicals” and “Oxidative stress biomarkers”, however, they were used with other secondary ones such as associated occupational exposure, agricultural pesticides, farmers, and rural workers through Boolean-type connectives (AND and OR). At first, the title and abstract of the articles found in the search that addressed biomarkers of oxidative stress and pesticides were considered adequate for the purpose of the study. However, studies published in English were used as definitive inclusion criteria, dealing with the relationship between exposure to pesticides, oxidative stress, and biomarkers. Thus, a total of 59 published articles were selected, which were used in this review. The references of these publications were checked for additional recent articles in relevant international journals to compile adequate information in this discussion.

THE USE OF PESTICIDES IN AGRICULTURE AND THE EFFECTS ON HUMAN HEALTH

Naturally, pests are a threat to agricultural production. Thus, for a long-time man controlled using a variety of natural substances with plant extracts and limestone (Warra, Prasad, 2020). However, with the industrial revolution, the world's population grew rapidly and became urbanized and globalized, requiring an unprecedented demand for agricultural products. In order to meet these needs, agribusiness began to significantly increase its production, investing in technologies, mechanization of processes, and extensive use of chemical compounds, especially agrochemicals (Carvalho, 2017).

Pesticides are chemical compounds used to control a variety of undesirable living organisms (Lee, Choi, 2020). They are used in different parts of the world for the protection of agricultural crops and in public health in the control of vectors or intermediate hosts of diseases (Dhananjayan, Ravichandran, 2018). Due to the great variety of these products, the classifications are variable, due to the different mechanisms of action, formulation, chemical groups, plant resistance, selectivity, and toxicological indices (Schaumburg *et al*., 2016). However, organochlorines, organophosphates, carbamates, pyrethroids, neonicotinoids, chloroacetanilides, aryloxyalkanoic acids, triazines, ureas, substituted glycine, triazoles, dithiocarbamates, benzimidazoles, and dicarboximides stand out (Kokolakis, 2019; Ledda, *et al*., 2021).

Due to the high biological activity of these compounds, and environmental persistence, their use can cause unwanted effects on human health and the environment (Dhananjayan, Jayakumar, Ravichandran, 2020; Wang *et al*., 2016). Incorrect handling of these phytosanitary products can result in severe acute poisoning, and in certain situations, adverse health effects can be seen in long-term and low-level exposures (Lebov *et al*., 2016).

The risk of exposure changes depending on the type of pesticide, route of exposure, duration, and health status of the individual. Contact with these compounds can cause biomolecular changes, resulting in the development of gastrointestinal, dermatological, carcinogenic, neurological, reproductive, and respiratory health problems or even death (Gangemi *et al*., 2016).

Adverse effects associated with exposure are observed in various groups of individuals characterized by different patterns and degrees of exposure. However, farmers and rural workers are considered the class with the highest risk of exposure, as they are involved in transporting, mixing, loading, and applying pesticides (Dhananjayan, Jayakumar, Ravichandran, 2020; Gangemi *et al*., 2016). Some of these individuals are aware of the risks related to the use of the product. However, the lack of training and equipment for safe handling increases health problems (Dhananjayan, Jayakumar, Ravichandran, 2020; Groot, Van't Hooft, 2016).

People exposed to pesticides may manifest acute symptoms resulting from exposure to high doses in a short period of time, such as irritation, nausea, vomiting, headache, salivation, and increased sweating, among others. Or the symptoms may come from chronic exposure, characterized by the appearance of late symptoms, through contact with small but continuous concentrations, causing damage that is often irreversible, such as neoplasms or paralysis (Jayaraj, Megha, Sreedev, 2016). Subacute intoxication is associated with moderate exposure, with subjective and vague symptoms (headache, malaise, stomach pain, weakness, and drowsiness) that may appear hours or days after exposure. The determining factors of risk of exposure are related to the non-use or inappropriate use of personal protective equipment and also to the high toxicity of the product (Gangemi *et al*., 2016).

As scientific knowledge about damage to health and the environment advances, new compounds are developed, tested, and marketed, with the aim of replacing products with high toxicity. Currently, there are more than 1000 active pesticide ingredients, among which the herbicide glyphosate represents more than 60% of the world market for non-selective herbicides (Borges *et al*., 2021; Mercado, Caleño, 2020).

THE USE OF BIOMARKERS IN THE RISK ASSESSMENT OF PESTICIDES

In general, exposure to chemical agents is estimated by measuring the concentrations of physical-chemical parameters in air, food, water, soil, dust, or other media with which a population or an individual is in contact (Maurya, Yadav, 2014). The use of these variables has some advantages in the evaluation study of occupational exposure to chemical agents by allowing the identification, quantification, and characterization of samples (Machado, Martins, 2018). However, due to the temporal and spatial irregularity of the samples, there is a momentary result of a dynamic situation (Kerfoot *et al*., 2021). Thus, biomarkers are used for biological monitoring as a tool in assessing the responses of biological communities to changes in original environmental conditions (Sumudumali, Jayawardana, 2021).

Biomarkers provide evidence that allows monitoring the physiological and biochemical impacts on the organism through the quantification of any biological substance or its biotransformation product, as well as any early biochemical alteration, whose determination in biological fluids, tissues, or emanated air, evaluates the degree of exposure and health risk, allowing estimation of exposure to a given compound and a possible correlation with the manifested effect (Dalmolin *et al*., 2020). They are employed in order to verify individual or population exposure to a given occupational situation or chemical substance, seeking to assess the risk when this is compared to an adequate reference (Dalmolin *et al*., 2020).

Biomarkers are applied for different purposes, which vary with the purpose of each study, and are classified as exposure, effect, and susceptibility biomarkers, which are considered tools that allow identifying toxic substances or adverse conditions that can promote health damage of biological organisms (Dalmolin *et al*., 2020; Machado, Martins, 2018). Thus, the choice of a biomarker requires that a series of requirements be met according to the study to be carried out, however, the appropriate biomarker must be able to reflect the exposure to the original compound, that is, be sensitive and vary according to the intensity. exposure / biological effect of the substance to the contaminant (Rietjens *et al*., 2018; Torres *et al*., 2018).

Blood and urine are the most used biological matrices in the quantification of biomarkers (Torres *et al*., 2018). However, there is a disadvantage in the use of blood because it is an invasive matrix, which can have an adverse effect on the study, its collection requires the participation of qualified people, in addition, storage and transport must be carried out properly, as the samples can degrade (Esteban-lópez *et al*., 2022). On the other hand, urine collection is accessible in large volumes and is not invasive, in addition to allowing the determination of very low concentrations of chemical substances, resulting from environmental exposure, mainly hydrophilic metabolites (Basu *et al*., 2018; Rousis *et al*., 2017). Other non-invasive matrices such as hair, breast milk, and nails have been used as biomarkers to assess environmental and occupational exposure (Torres *et al*., 2018).

OXIDATIVE STRESS AND EXPOSURE TO PESTICIDES

The health risk associated with exposure to pesticides is a consequence of disturbances in the antioxidant defense system. This is due to the imbalance between oxidant and antioxidant compounds, which causes the excessive generation of free radicals or decreases the speed of removal of these agents from the body, resulting in oxidative stress (Daenen *et al*., 2019; Gulcin, 2020).

The toxicity of pesticides is related to their biotransformation into metabolites responsible for the generation of reactive oxygen and nitrogen species (Jabłońska-Trypuć *et al*., 2017). ROS [Hydroperoxyl ($HOO^{\bullet}$), hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet -}$), and hydrogen peroxide (H_2O_2) are able to induce oxidation of nucleic acids, lipoproteins, cellular damage, and depletion of endogenous antioxidants. All this causes oxidation and loss of biological functions in the body (Garcia *et al*., 2022). This process of oxidative stress at the cellular level is illustrated in Figure 1.

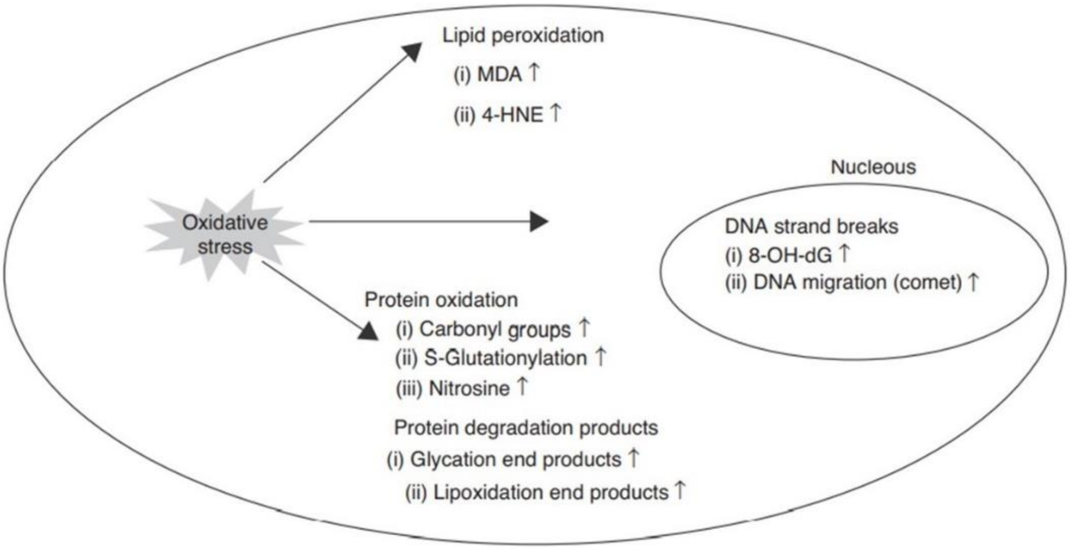

Figure 1- Representation of the process of occurrence of oxidative stress at the cellular level. Source**:** Lucio *et al*. (2018).

In the biological system, reactive species play important roles as they are associated with various processes such as the regulation of gene expression, cell proliferation, tissue development, phagocytosis, energy production processes, intercellular signaling, and synthesis of important biological substances (Sies, Jones, 2020; Zingg, 2019). However, the excess of these reactive compounds has harmful effects, as it promotes damage and death to macromolecules and cells, which can lead to the development of neurodegenerative diseases such as Alzheimer's and Parkinson's; cancer; cataracts; atherosclerosis; neoplasms; diabetes; gastrointestinal and respiratory inflammations (Sule, Condon, Gomes, 2022).

One of the main challenges of the human body is to maintain the balance between free radical production and antioxidant defense, as this stability has relevant health implications (Jabłońska-Trypuć *et al*., 2017). Thus, the body has developed several mechanisms to combat the damage caused by oxidative stress, the main one being the antioxidant system, formed by compounds or enzymes that delay or inhibit damage from the deleterious action of free radicals or non-radical reactive species, as they are stable enough to neutralize them by donating electrons (Sies *et al*., 2021).

Usually, in aerobic organisms, the oxidant system is divided into enzymatic and non-enzymatic (Jabłońska-Trypuć *et al*., 2017). Both protect the cellular structure and maintain adequate physiological concentrations of ROS in the body. Enzymatic antioxidants include the enzymes Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx), which often depend on different enzyme cofactors according to the cellular compartments of enzyme action

(Ighodaro, Akinloye, 2018; Radulescu *et al*., 2019). The non-enzymatic defense system is mainly composed of antioxidants of dietary origin, among which the following stand out vitamins, minerals, and phenolic compounds (Radulescu *et al*., 2019).

The activity of pesticides in the human organism has been correlated with the production of radical species and the oxidation of biomolecules, which is responsible for the genesis of several diseases. The presence of these chemical compounds was detected in the human placenta, in farmers, and in pregnant women, being responsible for weight loss, lipid peroxidation, and DNA damage in these individuals (Jabłońska-Trypuć *et al*., 2017; Sabarwal, Kumar, Singh, 2018). However, studies with mammals that directly associate the toxicity of pesticides and oxidative stress are still scarce, which may be evidence that such compounds have underestimated their action. Furthermore, most of the time, it is not possible to specify the exact mechanisms of action of these compounds in the human body, as they are not yet widely known, especially when it comes to environmental and occupational exposure. Thus, the specific biological effects depend on the chemical structure of the pesticide, which makes it reasonable to quantify them at the cellular level, as the results obtained can serve as an exposure indicator (Jabłońska-Trypuć *et al*., 2017).

BIOMARKERS OF OXIDATIVE STRESS USED IN THE ANALYSIS OF EXPOSURE TO PESTICIDES

When the production of reactive species exceeds the capacity of action of antioxidants, there is the oxidation of biomolecules, which generates specific metabolites that can be identified, measured, and quantified to provide information about the oxidative state of the organism, these oxidation by-products are called Biomarkers of oxidative stress (Maniaci *et al*., 2021). There are several methods available to quantify these markers. These include assays that target single molecules or groups of molecules, which differ in dynamics and can be formed through different independent biochemical reactions. Table 01 presents the main enzymatic and non-enzymatic biomarkers that have been used in the analysis of exposure to pesticides.

Oxidative stress biomarkers are mainly derived from the oxidation of lipids, proteins, and Deoxyribonucleic acid (DNA), with the former being the most expressive. In addition, another way to analyze oxidative stress is through indirect methods, based on antioxidant capacity (Garcia *et al*., 2022; Sies, Jones, 2020). Although different biological matrices such as saliva can be used in these measurements, the most used for clinical purposes are urine, blood, and their derivatives. However, urinary markers are not suitable for patients with kidney problems. Thus, the applicability of an oxidative stress biomarker depends on the choice of sample, which is defined by the characteristics of the individual (Peluso *et al*., 2017).

Table 1- Main oxidative stress biomarkers used in pesticide exposure studies

Biomarkers	Description	References
Superoxide dismutase (SOD)	It acts on the $O_2^{\bullet-}$ radical, dismutating it into H_2O_2, which is less reactive and can be metabolized by other enzymes.	Gonçalves *et al*., 2021
Catalase (CAT)	Transforms hydrogen peroxide into water and molecular oxygen.	Gonçalves *et al*., 2021
Glutathione Peroxidase (GPx)	Selenoenzyme whose action resides in the oxidation of glutathione to its corresponding disulfide GSSG, removing H_2O_2 and generating H_2O.	Gonçalves *et al*., 2021
Glutathione Reductase (GR)	Flavoprotein required to maintain glutathione in its reduced form and to control the NADP redox state in tissues where GSSG is available.	Gonçalves *et al*., 2021
Glutathione S-transferase (GST)	Group of enzymes that catalyze the formation of thioesters by the addition of GSH to a large number of compounds that contain an electrolytic carbon, with peroxidase capacity against organic hydroperoxides and the biotransformation of xenobiotics.	Gonçalves *et al*., 2021
Reduced Glutathione (GSH)	The only non-protein thiol present in species'erobic activities and its intracellular antioxidant role includes the detoxification of xenobiotics and ROS/RNS.	Gonçalves *et al*., 2021
8-hydroxy-2-deoxyguanosine	Product derived from the oxidation of deoxyribonucleic acid (DNA) used in the literature to assess damage from oxidative stress.	Costa *et al*., 2019
Ascorbate Peroxidases (APx)	Family of detoxifying enzymes that react with peroxides reducing them to water.	Gonçalves *et al*., 2021
Malondialdehyde (MDA)	Secondary metabolism product of polyunsaturated fatty acid peroxidation.	Sule; Condon; Gomes, 2022
3-nitrotyrosine	Product of tyrosine nitration mediated by reactive nitrogen species such as peroxynitrite anion and nitrogen dioxide.	Wigner *Al.*,2021
Vitamin C	It has the ability to neutralize radicals such as $O_2^{\bullet-}$ and $OH^{\bullet}$	Sule; Condon; Gomes, 2022
Vitamin E	It works by blocking the propagation step of lipid peroxidation of polyunsaturated fatty acids in membranes and lipoproteins, scavenging peroxyl radicals ($LO_2^{\bullet}$) faster than these radicals can react with adjacent fatty acids or with lipoprotein membranes.	Sule; Condon; Gomes, 2022

Source: Elaborated by the authors.

CONCLUSION

This study demonstrated that pesticides induce the production of RNS and ORS, which can cause oxidative stress in humans, which is related to several diseases. Thus, it was possible to verify that, although there are different proposals for biomarkers that can be used in clinical analysis to assess oxidative damage, the correct marker will allow a prognosis and correlation with the degree of exposure to the chemical agent. In addition, these tools have been increasingly used to estimate the risk of exposure and to set appropriate limits and concentrations for biological organisms in order to prevent toxic effects related to pesticides. Although they can be quantified in different types of biological matrices, there are some restrictions that are mainly related to the health conditions of the individuals, therefore, it is necessary to know the target population to correctly choose the marker and the sample to be used.

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