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Research Article

Evaluation of the effect of the pesticides glyphosate and chlorpyrifos on the activity of some neuronal enzymes using milk and turmeric in laboratory albino Wistar rats

Mareb A. Shawkat*
Department of Biology, College of Education, University of Samarra, Iraq
Wijdan I. A. Abd-alwahab
Department of Biology, College of Education, University of Samarra, Iraq

*Corresponding author. E-mail: maaribalbbasi@gmail.com

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Absrtact

Chlorpyrifos and glyphosate are the most commonly used pesticides that have harmful effects on oxidative stress and neuroenzyme disorders. The current research aims to evaluate the effect of pesticides on acetylcholinesterase (AChE), malondialdehyde (MDA) and glutathione (GSH) in laboratory rats to study the protective effect of turmeric and milk against the effects of the used pesticides. The study was conducted inthe General Laboratory of Pharmaceuticals of the Pharmaceutical Industries Company in Samarra from the beginning of January to the end of February 2023, using 45 albino wistar rats aged 3-4 months and weighing 160-200 grams. The Groups were divided into nine Groups, each containing 5 animals: the control Group, the Groups exposed to pesticides, and the Groups treated with turmeric and milk. The results showed a significant increase ($P \leq 0.05$) in MDA(3.819 ±0.831), AChE (116.897 ±13.42) and a significant decrease in GSH (1.293 ±0.197) in the Groups exposed to pesticides compared to the control Group. Treatment with milk and turmeric led to a significant decrease ($P \leq 0.05$)in the concentrations of MDA and AChE in the treated groups compared to the Groups exposed to pesticides only. While treatment with milk and turmeric led to a significant increase ($P \leq 0.05$) in GSH in the Groups exposed to chlorpyrifos and treated, and no significant difference was shown in the Groups exposed to glyphosate and treated compared to the Group exposed to glyphosate only. We conclude that turmeric and milk have a therapeutic effect in removing the harmful effects on the nervous system and reducing oxidative stress.

Keywords: Chlorpyrifos, Glutathione, Glyphosate, Malondialdehyde, Neurol enzyme

INTRODUCTION

Exposure to pesticide combinations leads to dangerous problems for the environment and human health. Many researches have demonstrated that Pesticides have been widely used on crops in recent years, which has led to many environmental damages, especially water and soil pollution The most widely used pesticides with human and environmental concerns were organophosphates (Pathak *et al*., 2022). The human body was exposed to pesticides either directly or indirectly, causing severe reactions such as headache, irritation, vomiting, sneezing, and rashes on the skin and affecting the respiratory system. The severity of these pesticides on humans depends on the time of exposure and concentration. In general, the consumption of such vegetables and fruits grown in soil and water contaminated with pesticides that have been used for a long time leads to an increase in the concentration of toxins inside the body organs and causes chronic diseases such as neurotoxicity, cancer, asthma, reproductive disorders, heart disease, diabetes, etc. (Kalyabina *et al*., 2021). the relationship between contact with pesticides and the occurrence of many endocrine disorders, harmful outcomes, birth defects, neurological, respiratory, and hepatic, blood, and immune effects, in addition to the development of diseases.There were a large number of types of pesticides, exceeding 1000 types. These pesticides differ in properties and toxicity and have been classified according to their toxicity (Kumar and Kumar,2019), chemical composition, target pest, environmental stability, method of use, and nature of the preparation (Kaur *et al.*, 2019).

Pesticides have been used to eliminate pests and limit their spread, but the random and unregulated use of these pesticides causes harmful effects on human health (Kaur *et al*., 2024). Annually, pesticides cause more than 3 million cases of poisoning and 220,000 deaths, and 50% of those infected with poisoning and 25% of those who died ate food contaminated with pesticides (Singh *et al*., 2017). Contamination can occur by drinking water, breathing air, eating contaminated food, and skin contact (Kim et al., 2017). Oxidative stress, an imbalance in favor of oxidants over antioxidants a major contributor to the development of many common diseases that negatively affect humans. Counteracting these oxidants has been an effective strategy to reduce levels of oxidative stress in living organisms (Muscolo *et al* ., 2024). Living cells continuously produce free radicals or reactive oxygen species (ROS) through the respiratory chain and during energy metabolism. ROS can be harmful or play important physiological roles in our bodies. In addition to being produced during normal cell metabolism, external factors, such as radiation and air pollutants, such as pesticides, can also produce ROS.

The human body has various intrinsic mechanisms to combat oxidative stress by producing antioxidants obtained from foods and vegetables. When the number of free radicals increases, their accumulation in the body generates a phenomenon called oxidative stress. This oxidative damage leads to the development of late-onset diseases caused by ROS/ROS in any system of the body and can lead to clinical conditions such as cancer, arthritis, atherosclerosis, and neurodegenerative diseases (Sharma, 2014). The nervous system is a major target for pesticides and organophosphates' acute and chronic effects. Neurotransmitter enzymes play a crucial role in all central and peripheral nervous system functions, regulating nervous system activity and maintaining the balance of various vital processes (Martínez *et al*., 2018). One of the most important of these enzymes for the transmission of nerve signals is acetylcholinesterase (AChE): exposure of the nervous system to toxins or pesticides negatively affects the activity of the enzyme, leading to the accumulation of acetylcholine and causing neurological disorders, as it is responsible for the breakdown of the neurotransmitter acetylcholine after the transmission of nerve signals between cells. It also plays an important role in preventing excessive nerve activity and ending nerve stimulation. A second type of enzyme is considered to protect the nervous system from nerve stress: glutathione peroxidase (GPx), which protects neurons from oxidative damage by removing free radicals. Superoxidase (SOD): converts harmful free radicals into less harmful substances, helping to prevent oxidative stress in the brain and neurons. Its deficiency has been linked to diseases such as Parkinson's and Alzheimer's (Ashkan *et al*., 2021). The third type of enzymes regulates the levels of neurotransmitters, such as catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO), which regulate the levels of transmitters in the brain, and the fourth type of enzymes maintains the integrity of nerve membranes, such as myelin lamellar protein (MBP) whose function is to maintain the integrity of the myelin sheath which plays an important role in the speed of transmission of nerve signals because it surrounds nerve fibers and any defect in it leads to damage to myelin as occurs in multiple sclerosis. Type V enzymes that generate cellular energy such as neuronal glucokinase contribute to the consumption of glucose in the brain and provide the energy needed for various neuronal processes, and type VI neuronal enzymes contribute to the neuronal balance of biochemical processes in the brain such as the conversion of dopamine to norepinephrine by dopamine beta hydroxylase, as this balance is necessary for regulating motor responses (Alizadeh Pahlavani, 2024; Yang *et al*., 2023).

Given the importance of these enzymes and their effects, the present study highlights the danger of repeated exposure to these pesticides on public health, which necessitates the need to take preventive measures to reduce human and environmental exposure to these compounds, especially the reason for conducting the study is the widespread use of pesticides, chlorpyrifos and glyphosate. A series of studies indicate that glyphosate can the human nervous system, These investigations have shown that it can penetrate and affect the blood-brain barrier and cause different types of short-term or long-term disorders (Costas-Ferreira *et al.,* 2022). In the human nervous system, maintaining the balance of these neuronal enzymes is essential to ensure the safety of neurons and reduce the risk of neurological diseases. This study aimed to know the effect of organophosphate pesticides on the enzyme (ACH), the level of (MDA) and the level of (GSH), in addition to studying the effect of turmeric and milk individually for each and their effect together in confronting the damage caused by oxidative stress resulting from continuous exposure to pesticides.

MATERIALS AND METHODS

Materials

Preparing turmeric and milk doses

Turmeric rhizomes were purchased from the local markets of Samarra city and cleaned from impurities and dust. They were then ground using a special electric grinder and stored in tightly sealed bottles at room temperature until use. And whole milk was of high quality and distinguished by the Saudi industry due to its abundance in the local markets and ease of obtaining it. Turmeric powder was used in a dose of (20 mg/kg) mixed into 2.5 ml of distilled water, and a milk solution was used in a dose of (2 ml/kg) and prepared in a single daily oral dose for 15 days.

Experiment Design

The animals under the study (45) were albino rats of the Wistar rat type purchased from the animal house in the Uwainat area. divided into 9 groups, each Group containing 5 animals with similar weights (160- 200) germ. Group I (without any treatment) was the control group and the other eight were considered treatment groups (Group II to Group IX. Group I (control): Rats were given only water and food over the experiment period. Group II: The rats were sprayed with chlorpyrifos for 10 days, while Group III was also exposed to the pesticide for 10 days but treated with turmeric by dosing for 15 days. The Group IV was sprayed with chlorpyrifos for 10 days and treated with milk for 15 days. Group V was sprayed with the pesticide chlorpyrifos for 10 days and treated with milk and turmeric for 15 days. Group VI was exposed to glyphosate for 10 days, while Group VII was exposed to glyphosate by spraying for 10 days and given turmeric doses for 15 days. Also, Group VIII was sprayed with glyphosate for 10 days, but was treated with milk doses for 15 days. Finally, Group IX was sprayed with glyphosate for 10 days and treated with both milk and turmeric doses for 15 days.

After the specified period of the study, 6 ml of blood sample was drawn from the jugular vein of each albino rat. Two ml of each drawn blood was put in an EDTA tube for Complete Blood Count (CBC) tests. The serum was separated from the remaining blood to conduct the neuronal enzymes acetylcholinesterase, malondialdehyde, and glutathione.

Analysis of enzymes

The activity of the enzyme acetylcholinesterase (AChE) was measured In serum addition to its effect on the nervous system, the concentration of the peroxidation level of lipids in the amount of malondialdehyde (MDA) and the concentration of the antioxidant Glutathione (GSH) using a measuring kit supplied by USCN (USA). As stated in the manufacturer's instructions. The examination was performed using the (ELISA) technique, which is based on antigen-antibody interactions. The plate is pre-coated with an antibody to human MDA, ACH, GSH. (MDA, ACH, GSH) present in the sample is added and binds to the antibodies coated on the wells. Then the biotinylated (MDA, ACH, GSH) antibody is added and binds to MDA, ACH, GSH in the sample. Then the horseradish peroxidase (HRP) enzyme solution is added and binds to the biotinylated (MDA, ACH, GSH) antibody. After color transformation, the absorbance is measured at a wavelength of 450 nm.

Statistical analysis

The study results were analysed using analysis of variance (ANOVA). Also, the arithmetic means of the coefficients were then compared using Duncan's Multiple Range test with a significance level of ($P \leq 0.05$) and using the statistical program SPSS (Duncan, 1955).

Ethical approval

This study was conducted in accordance with academic laws, requiring clearance from the Ethics Committee of Healthy Minsitry dated 25/10/2023 and Numbered 35/2023036 and University of Samarra dated 8 /1/ 2023 and Numbered 3/7/40 .

RESULTS AND DISCUSSION

The results in Table 1 show that there was a significant increase ($P \leq 0.05$) in the concentration of the neuronal enzyme acetylcholinesterase (AChE) and malondialdehyde (MDA) in two groups exposed to pesticides (glyphosate-exposed Group) and (chlorpyrifos-exposed Group) only in comparison with the control group. While a noticeable decrease ($P \leq 0.05$) was noted in the concentration of AChE and MDA in the (groups exposed to the pesticide's glyphosate and chlorpyrifos treated with turmeric and milk) compared to the Group exposed to glyphosate and chlorpyrifos) only. As for the results in Table 1, they show that there was a significant decrease ($P \leq 0.05$) in the concentration of glutathione (GSH) in the (groups exposed to the pesticides glyphosate and chlorpyrifos) only compared to the(healthy control group), while a significant rise in the concentration of GSH was observed in the (Group exposed to glyphosate and treated with turmeric) only. The rest of the groups do not show a significant difference compared to th ose exposed to pesticides. However, a significant increase in GSH concentration was observed in all groups exposed to chlorpyrifos and those treated with turmeric and milk, compared to the Group exposed to chlorpyrifos only.

The high concentration of the neuroenzyme in the present study may be attributed to direct exposure to pesticides, as the concentration in the second Group exposed to chlorpyrifos (114.170 ± 8.308) ng \ml and the sixth Group exposed to glyphosate (116.897 ± 13.42) ng \ml compared to the first Group of healthy controls (30.369 ± 0.103) ng \ml as mentioned in the table, and the mechanism by which the neurotransmitter enzyme acetylcholinesterase affects brain functions, and any changes in the enzyme activity. It was accompanied by signs of behavioral neurotoxicity, and thus the concentration of acetylcholinesterase in the body can be considered a neurotoxicity that refers to the direct or indi-

Table 1 . Concentration of the neuronal enzyme acetylcholinesterase, malondialdehyde, and glutathione exposed to the two pesticides in all studied groups

Groups	**Acetylcholinesterase (AChE) ng/ml**	**Malondialdehyde (MDA) μmole /L**	**Glutathione (GSH) μmole / L**
Group 1 (Control, without any treatment)	30.369 ±0.103C	1.108 ±0.176Cd	65.694±2.297A
Group 2 (Chlorpyrifos)	114.170 ±8.308A	4.975 ±0.663A	2.683 ±0.562Ef
Group 3 (Chlorpyrifos treated with turmeric)	23.282 ±1.319 C	0.514 ±0.103E	33.221 ±0.904C
Group 4 (Chlorpyrifos treated with milk)	22.776 ±2.716C	0.572 ±0.082	40.773 ±5.484B
Group5 (Chlorpyrifos treated with milk and turmeric)	23.064 ±3.395C	0.854 ±0.128De	20.568 ±1.690D
Group 6 (Glyphosate)	116.897 ±13.42A	3.819 ±0.831B	1.293 ±0.197F
Group7 (Glyphosate and treated with turmeric)	63.280 ±2.854B	1.389 ±0.139C	5.544±1.581E
Group8 (Glyphosate treated with milk)	62.498±6.358B	1.487 ±0.267C	3.809 ±0.785ef
Group 9 (Glyphosate and treat ed with milk and turmeric)	61.107±6.047B	1.425 ±0.328C	3.409 ±0.356ef

Values show Mean ± Standard deviation S.D; The different letters (vertically) indicate the presence of significant differences at a significant level ($P \leq 0.05$)..

rect effect of chemicals that affect the nervous system of humans or animals (Milatovic *et al.,* 2006; Schliebs and Arendt, 2011). Emerging evidence suggests that toxic effects on physiological systems outside the brain of the nervous system, especially the immune system, the endocrine system, and the gut microbiome, may negatively affect chemicals that cause neurotoxic diseases in humans, many of which were used as experimental tools to explore physiological and pathological processes in the nervous system of animal tissues in vivo and in vitro (Spence and Lein, 2024).

It's noted that the concentration of malondialdehyde rises in the groups exposed to the pesticide's glyphosate and chlorpyrifos and in the treated groups. Its level particularly increases in the treated groups of glyphosates, unlike the treated groups of chlorpyrifos. It was an indicator of oxidative stress that resulted in the generation of free radicals. Free radicals increase the lipid oxidation reactions that occur in the cell membranes and the subsequent partial loss and damage of elasticity through the process of lipid peroxidation. Consequently, it leads to a rise in MDA concentration (Chaudhary *et al*., 2023). The unsaturated fatty acids of cell membranes were considered the part most exposed to free radical reactions because they possess double bonds. And, the continued generation of free radicals results in forming lipid peroxidation. The absence of antioxidants, whether from internal or external sources, results in oxidative damage to which various body tissues were exposed.Therefore, measuring the level of MDA in the blood and tissues indicates the extent to which body lipid was exposed to oxidation, as its concentration increases in cases of oxidative stress resulting from many diseases (Ungvari *et al*., 2018).

Based on experimental evidence, different types of pesticides appear to have a common effect, which is to induce pesticide-induced oxidative stress due to both reactive oxygen species (ROS) and reactive nitrogen species (RNS), which have been associated with many diseases including neurodegenerative diseases, cancer, inflammation, and cardiovascular diseases. ROS and RNS can activate at least five independent signaling pathways including mitochondrial apoptosis. Limited in vitro studies suggest that exogenous antioxidants can reduce or prevent the harmful effects of pesticides (Sule *et al* ., 2022). Human exposure to them due to largely indiscriminate use was associated with health problems including neurotoxic changes. High levels of pesticide residues and their metabolites have been reported in food ingredients and products. Epidemiological studies suggest that human exposure to pesticides may be a significant risk factor for neurological disorders, including Parkinson's disease and Alzheimer's disease. In light of the increased risks to human and animal health from exposure to pesticides that cause oxidative stress, current studies have focused on pesticide-induced neurochemical changes and associated behavioral disorders in farm workers, which may demonstrate a potential link between exposure and associated health risks (Kori *et al*., 2018).

The results of a study conducted on rats poisoned with CPF and treated with vitamin C showed a decrease in lipid peroxidation and GST activity, and an increase in GSH (Aly *et al*., 2010). levels. Their study indicated that vitamin C could be an important nutritional component based on its ability to mitigate pesticide toxicity on the nervous system and liver (Otuechere *et al*., 2012). All of these studies indicate a clear protective role of vitamin C against pesticide-induced toxicity. Therefore, it is likely that other compounds with antioxidant properties could also have significant beneficial effects against pesticide-induced toxicity, suggesting that vitamin C was able to provide significant protection against pesticide toxicity. This was consistent with the present study when using turmeric and milk in treatment due to the antioxidants in turmeric and the many benefits of milk, which showed significant increases in the treatment groups exposed to chlorpyrifos with turmeric and milk, which may mitigate the resulting oxidative damage,The decrease in the level of glutathione (GSH) in groups of exposed rats may be due to the increased consumption of antioxidants to counter the production of excess free radicals during cases of excessive use of pesticides permanently and randomly. Thus it leads to the generation of free radicals. Another interpretation of its decrease in concentration could be due to an increase in the rate of its consumption in the body's cells. This was caused by one of the most essential non-enzymatic antioxidants, which mainly eliminates free radicals and their products (ROS). Furthermore, oxidative stress causes glutathione oxidation because of its antioxidant effect. In this case, it was converted to the oxidized form of glutathione disulfide (GSSG) It was toxic and works to stimulate the formation of new types of free radicals. Also, large amounts of glutathione were consumed due to its role in enhancing the effectiveness of certain antioxidants, like vitamin C, in cases of oxidative stress. The level of glutathione, as a result of its consumption, can be influenced by other elements such as hormonal balance, degree of growth, age, nutritional status, and the level of the glutathione production process within the cell (Diaz-Del Cerro, Martinez de Tod, Félix, Baca, and De la Fuente, 2023).

The concentration of glutathione in the body is a vital marker and therapeutic target for many chronic and age-related diseases. It can negatively affect the production of glutathione in the body. As a result of several reasons and factors, including unhealthy lifestyles and diets, all of which contribute to reducing glutathione levels and due to the increased production of free radicals, which increases the deterioration and decrease of glutathione in the body due to oxidative stress resulting from environmental factors such as pesticides, pollutants, radiation and exposure of the body to environmental toxins and heavy metals, all of these things increase the deterioration of low glutathione levels. It has been hypothesized that human glutathione levels can be improved by using fruits and vegetables containing glutathione or amino acids that help synthesise (glutathione). Glutathione plays a pivotal role in physiological processes, including maintaining the oxidation-reduction balance, reducing oxidative stress by promoting metabolic detoxification of both foreign and internal compounds, and regulating the function of the immune system. Glutathione depletion leads to the formation of free radicals, inhibition of some cell functions, and the release of inflammatory cytokines, all of which were known to cause many chronic degenerative diseases and loss of function with aging. Furthermore, dysregulation of glutathione metabolism has been shown to cause central nervous system diseases, weakness, muscle fragility, inflammation, chronic liver diseases, metabolic diseases, lung diseases, and cardiovascular diseases (Novelli and Bianchetti, 2022). Knowing the concentration of glutathione makes it possible to detect early diseases due to its important role in regulating cellular stress and its importance in reducing these diseases. The intake of nutrients, including antioxidants and glutathione, has been recommended (Teskey *et al* ., 2018) . The reason for choosing turmeric in our study that it was considered one of the antioxidants that showed positive results in increasing the level of glutathione in groups exposed to chlorpyrifos treated with turmeric and milk.

Exposure of rats to pesticides leads to a rise in MDA and AChE concentration and concurrently a decrease in GSH concentration compared to the control group. This result was consistent with the results of the current study and the results of (Stenvinkel *et al*., 2004). In a similar context, recent laboratory studies have revealed that turmeric was one of the most powerful antioxidants at different action levels. It was anti-inflammatory, anti-bacterial, and anti-microbial. Sufficient levels of turmeric and milk can be consumed to ensure adequate protection from oxidation factors, which can reduce the harmful effects of exposure to these pesticides. It can be easily obtained because turmeric is widely available and popular in the markets and is considered a daily spice and easy to obtain and does not require complex preparations to consume, it is incorporated into the daily diet in various foods in addition to the health benefits of enhancing immunity, anti-inflammatory and improving digestion, which makes it a natural treatment that is easy to include in daily life. (Harifi-Rad *et al*., 2020). It is considered an anti-toxin through daily consumption (Hewlings and Kalman, 2017). In addition, milk contains all the B vitamins and ascorbic acid, one of the basic antioxidants soluble in water and milk. Vitamins A and E were the basic antioxidants soluble in milk and fat and were considered a treatment for poisoning. Milk alone will not be sufficient to treat this problem. Rather, it was placed within the first aid category if anyone became poisoned. It helps alleviate the severity of poisoning as a preventive substance to reduce poisoning and its level in the stomach. It works to form an insulating layer for the stomach (Idowu *et al*., 2022; Khan *et al*., 2017).

The high pharmacological potential of medicinal herbs as natural antioxidants and anti-inflammatory agents suggests that to determine the efficacy of natural plant products as chemopreventive agents present in foods consumed by humans, the use of these additives to mitigate the effects of glyphosate herbicides was considered (Yousefi *et al*., 2021). The use of natural plant products in our study was because they are considered antioxidants against oxidative stress upon exposure to pesticides and most of the products and herbs are available. The inclusion of ginger in the diet significantly improved growth performance, digestive health, immune and inflammatory responses and is considered an antioxidant. Turmeric and milk outperformed ginger in this study when compared to ginger. Both are natural plants used in traditional medicine to treat the body from toxins, including those present in pesticides. However, each has unique properties that affect how it interacts with the body. Both have anti-inflammatory properties, but ginger effectively calms acute inflammation, while turmeric is more beneficial in chronic conditions. It also enhances liver function and deep detoxification, as it contains curcumin, which was more effective in reducing oxidative stress that occurs when exposed to pesticides (Fuloria *et al*., 2022; Abdelmagid *et al*., 2022).

Conclusion

The results of the present study concluded that organophosphorus pesticides, namely glyphosate and chlorpyrifos, had significant effects on the activity of the neurotransmitter enzyme (AChE), GHS, and MDA levels in the studied samples. There was a decrease in the activity of the neurotransmitter enzyme and an increase in the levels of MDA, indicating oxidative stress. In addition, a decrease in the levels of glutathione was recorded, indicating the negative effect of these pesticides on the internal antioxidant system. The effect of treatment with turmeric and milk was studied, and the results showed a significant improvement in resistance to oxidative stress and a reduction in damage from pesticide exposure. Turmeric, in particular, showed greater effectiveness in enhancing antioxidant defenses by raising glutathione levels and reducing MDA content. The combined treatment with turmeric and milk showed positive effects, but the effectiveness of turmeric alone was slightly more pronounced. These results highlight the therapeutic potential of turmeric as a protective agent against damage resulting from pesticides. Based on these results, further research can be recommended to explore how natural remedies, such as turmeric and milk, affect pesticide poisoning and to develop effective treatment strategies to reduce their negative effects on human health and the environment.

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Conflict of interests

The authors declare that they have no conflict of interest.

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