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Effect of Nano-magnesium oxide (MgO) NPs on some biochemical characteristics in male rats exposed to bisphenol A

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***Abstract*:**

This study was conducted to determine the effect of oral administration of two concentrations (30, 40)% of Nano magnesium oxide, where the sol-gel method was used to synthesize Nanoparticles of magnesium oxide, where Magnesium nitrate hexahydrate $Mg(NO_3)_3 \cdot 6H_2O$, deionized water, and Caccinia macranthera plant extract were used as raw materials for magnesium, solvent, and stabilizing agent, respectively. In the relative weight of some internal organs, red blood cell parameters, white blood cell counts, lymphocyte and monocyte ratios, and immunoglobulin values in male albino rats exposed to bisphenol A, Bisphenol-A caused a significant decrease ($P \leq 0.05$) in the weight of the liver, spleen, and kidneys, hemoglobin concentration (Hb), red blood cell count (RBCs), packed cell volume (PCV), platelet count, lymphocyte and monocyte ratios, immunoglobulin values, and a significant increase in white blood cell count (WBCs). While a significant increase ($P \leq 0.05$) was observed in the weight of the liver, spleen and kidneys, the concentration of hemoglobin Hb, the number of red blood cells RBCs, the packed cell volume PCV, the number of platelets, the percentage of lymphocytes and monocytes, and the values of immunoglobulins, and a significant decrease in the number of white blood cells WBCs when treated with Nano-magnesium oxide.

***Keywords*: Nano magnesium oxide, bisphenol A, Kidney, Liver.**

1. Introduction

Nanoparticles are small materials with properties that make them suitable for new applications of great importance in commercial and medical developments (with very small dimensions less than 100 Nanometers). In recent years, developments in the field of Nanotechnology have been accompanied by Nano-sized particles used in medicine, therapeutics, industry, synthetic textiles, and food packaging. Medicines have recently developed in several ways, as Nanoparticles have been used to treat many diseases, including cancer. These particles are characterized by their ability to provide controlled drug delivery, enhance permeability, and target the tumor. In addition, Nanomedicines interact with cancer-causing cells and enter them in an easy way (Ali, 2023).

Nanostructured oxide materials have been widely studied due to their large surface area, unusual absorption properties, few defects, and rapid diffusion ability. In addition to their stability under harsh conditions, they have been observed to be safe materials for humans and animals. Nanoparticles exhibit new properties and characteristics due to their small size, which are very different from their non-Nano or regular counterparts. Magnesium oxide (MgO) is a solid material with unique properties, high ionic character, crystalline structure, simple stoichiometry, surface structural defects and numerous applications in many fields such as electronics, petrochemical products, absorption, ceramics, reflective and anti-reflective coatings and treatment of chemical waste (Bindhu et al., 2016).

Nano magnesium oxide has good biological activity capabilities and biocompatibility, and magnesium ions do not contain toxic substances (Bader et al., 2021).

Over the past few decades, researchers have been increasingly interested in monitoring and understanding the harmful effects of environmental pollutants such as pesticides, herbicides, and industrial wastewater all over the world (Abdul Ghaffar et al., 2020). Many of these chemicals have been used in many applications such as agriculture, industries, public health management, environmental protection, and aquatic life (Rubin et al., 2019).

Many of these synthetic compounds have adverse effects on the endocrine system (Scarano et al., 2019) and cause disruption of multiple organ functions in humans and animals (Saima et al., 2021). One of the most endocrine disrupting chemicals is bisphenol A, which causes harmful effects on the pituitary gland (Chen et al., 2017), gonads (Wang et al., 2019), and brain (Molina et al., 2018).

It is considered highly toxic to organisms, so at present it has been found to have an effect on human health, so it is important that human health and environmental cleanliness are priorities that necessitate finding quick and effective ways to reduce bisphenol, so many methods have been used to decompose and reduce the toxicity of bisphenol in recent years, such as absorption, biodegradation and photocatalysis (Yin et al., 2021).

1.1 Research Problem

The main research problem lies in the risks resulting from the accumulation of bisphenol A in food, which is transferred from food storage containers directly to the food, posing a threat to human health and causing significant damage to various organs of the human body, such as the kidneys, liver, and spleen, as well as damage to the blood and damage resulting from a defect in the body's immunity.

1.2 Research Objectives

The research aims to use modern and low-cost technologies to reduce the risk of bisphenol A by reducing its accumulation in the internal organs of the body by removing it from the human body. Nanotechnology, represented by nano magnesium oxide, was used to remove the risk of this substance from the human body.

2. Related Work

Recent studies have indicated that bisphenol A is an industrial and commercial chemical widely used in the manufacture of beverage containers, epoxy resins, and polyethylene plastics (Lan et al., 2017; Chen et al., 2017). Bisphenol A is frequently detected and has become a serious health problem due to its ubiquitous presence in the environment, food, and drinking water (Djordjevic et al., 2019). Recent research and studies have shown the toxic effects of bisphenol on physical and behavioral factors such as loss of movement, loss of appetite, and the appearance of molecular abnormalities in various tissues such as the liver, brain, kidneys, and reproductive organs of animals (Kumari and Khare, 2018).

3. Methodology

A study of the toxic effect of Nano magnesium oxide was conducted on laboratory animals, 3 rats for each concentration, in addition to the control group, which was given water and natural food for 48 hours. It was found that it was non-toxic within the two mentioned concentrations (30%, 40%), as no toxic symptoms were observed for Nano magnesium particles during the test period to determine the effective dose or the occurrence of deaths in laboratory animals for different concentrations. This indicates the non-toxicity of this substance if used within the dose specified by the test.

This experiment was conducted in the animal house of the College of Veterinary Medicine and the laboratories of the College of Agriculture / Tikrit University, where 20 adult male albino Sprague-Dawley weanling rats were used, aged 9 weeks and weighing between 200-220 grams, divided into 4 groups, each group containing 5 male rats, which were distributed randomly and included the following:

1) The first group (M1): The negative control group (healthy) animals that were given standard food and drinking water without any additives.

2) The second group (M2): The infected group that were given 2 ml. Bisphenol A at a concentration of 10 mg/kg animal/day.

3) The third group (M3): The group of infected animals that were given 2 ml. Bisphenol A at a concentration of 10 mg/kg animal/day + 2 ml. MgO NPs at a rate of 30%.

4) The fourth group (M4): The group of infected animals that were given 2 ml. Bisphenol A at a concentration of 10 mg/kg animal/day + 2 ml. MgO NPs at a rate of 40%. The purpose of using two concentrations is to know which one is more effective.

The male laboratory rats were given the materials studied above after mixing them with distilled water (Rantala, 1974). After dissolving them in the concentrations shown in the groups above. The initial weight of the rats was taken after one day of feeding them individually at a temperature of 20-25ºC and lighting for at least 12 hours per day. The food was provided open as it was prepared according to what was mentioned by NAS- NRC, (2002). During the experiment period that lasted for 28 days.

After the end of the specified period of the experiment, the laboratory rats were starved immediately for 20 hours, after which they were anesthetized using chloroform, then they were dissected from the chest area and blood was drawn from the heart to conduct the necessary examinations. Approximately 3-4 ml. of blood was drawn using a syringe and placed in two tubes, the first containing an EDTA anticoagulant to measure the CBC, while the other contained approximately 6 ml. of blood and was free of anticoagulant. The two tubes were centrifuged using a centrifuge at a speed of 3000 rpm for 15 minutes to obtain the serum, which was stored at a temperature of -20 ºC until laboratory tests were conducted on it, as indicated by (Burtis et al., 2021). Laboratory animals were also dissected to obtain their internal organs, including the liver, spleen and kidneys, and weighed on a sensitive scale.

Blood collected using a test tube containing EDTA anticoagulant is used to measure the parameters of red blood cells (RBC) (106/mm3), hemoglobin concentration, packed cell volume (PCV), platelets, total white blood cell count (106/mm3), the percentage of monocytes and lymphocytes using the complete blood count system using the Biolabo France kit and the analysis was done using the Japanese Shimadzu spectrophotometer and according to the wavelength specified by the manufacturer of each analysis and the concentration was calculated according to the equation and according to the kit company leaflet according to the method mentioned by (Imtara et al., 2018).

The data were analyzed statistically through the experimental system within the ready-made statistical program (SAS, 2001) and using the complete random design system CRD, as the averages were chosen according to what was stated in Duncan's (1955) multiple range test to determine the significance of the differences between the averages of the factors affecting the studied traits at the significance level ($P \leq 0.05$).

4. Result Discussion

Effect of oral administration of MgO NPs on the relative weight of some internal organs in rats:

Table 1 shows the effect of oral administration of Nano magnesium oxide MgO NPs on the weight rates of the liver, spleen and kidneys of the rat groups treated with bisphenol for 28 days. The results showed a significant decrease ($P \leq 0.05$) in the weight rates of the internal organs of the rat groups treated with bisphenol M2, which reached 6.47, 1.26, 1.13 g/100 g of body weight, respectively, compared to the healthy control group M1, which was 9.81, 1.93, 2.45 g/100 g of body weight, respectively. The results also showed a significant increase in the weight rates of internal organs when adding Nano magnesium oxide at the above concentrations with bisphenol compared to group M2 to which bisphenol was added alone.

Table 1: The effect of oral administration of MgO NPs on the relative weight of some rat organs (g/100 g body weight) after 28 days of care.

Treatments	Liver	Spleen	Kidney
M1	a 0.5 ±9.81	a 0.06 ± 1.93	a 0.06 ± 2.45
M2	d 0.06 ± 6.47	ab 0.05 ± 1.26	c 0.05 ± 1.13
M3	c 0.5 ±7.73	ab 0.04 ± 1.51	ab 0.06 ± 2.07
M4	b 0.45 ± 8.94	ab 0.05 ± 1.87	ab 0.5 ± 2.29

Different letters in the same column indicate to significant differences at (P≤0.05).

The significant decrease (P ≤ 0.05) in the weight rates of internal organs when bisphenol A was consumed by laboratory animals is due to the toxic effect of this substance on the liver, spleen and kidneys, which causes an effect on fat metabolism in male rats (Chao et al., 2020).

These results are consistent with what was stated by (Sara et al.,2021) that Nano magnesium oxide has a noticeable effect in increasing the weights of the internal organs of laboratory animals. (Bhanuramya et al., (2017) stated that the reason for the increase in the weight of the internal organs of laboratory animals is the result of the accumulation of Nano magnesium oxide in these organs during the experimental period.

Effect of oral administration of MgO NPs on red blood cell parameters of rats:

Table 2 shows the effect of oral administration of MgO NPs on the number of red blood cells of rats treated for 28 days. The results showed a significant decrease (P≤0.05) in the concentration of hemoglobin, red blood cell count, packed cell volume (PCV) and platelet count of rat groups treated with bisphenol M2, reaching 9.93 g/dL.

3.56 (×106/mm3), 31.77%, (103/mm3) 201 respectively compared with the healthy control group M1 whose parameters were 12.32 g/dL, 6.41 (×106/mm3), 44.95%, 409 (103/mm3) respectively. While a significant increase in the above parameters was observed when treated with Nano magnesium oxide in groups M3, M4, as the hemoglobin was 10.91, 11.87 g/dL, the red blood cell count (RBCs) was 4.94, 5.98 (×106/mm3), the packed cell count (PCV) was 39.83, 42.13%, and the platelet count was 358.5, 367.4 (103/mm3) respectively compared to the rat groups treated with bisphenol (M2).

Table 2: Effect of oral administration of MgO NPs on red blood cell parameters of rats after 28 days of care.

Treatments	Hb (g/dl)	RBCs($\times 10^6$/mm^3)	Hem. (PCV) %	Plat. (10^3/mm^3)
M1	a 0.05±12.32	a 0.05± 6.41	a 0.5±44.95	a 0.05±409
M2	c 0.20± 9.93	c 0.04±3.56	d 0.05±31.77	d 0.06±251
M3	b 0.17±10.91	b 0. 6± 4.94	c 0.5±39.83	b 0.5±358.5
M4	ab 0.46±11.87	ab 0.05± 5.98	ab 0.6±42.13	a 0.06±367.4

Different letters in the same column indicate to significant differences at (P≤0.05).

The significant decrease in red blood cell parameters in laboratory animals is due to the disturbance in the formation of these cells as a result of exposure to bisphenol (Sanghamitra et al., 2017). In addition, bisphenol causes mitochondrial dysfunction (Asahi et al., 2010). The decrease in red blood cell parameters and the decrease in blood hemoglobin may be due to hemolysis resulting from reactive oxygen species generated by bisphenol (Hager et al., 2020).

These results are consistent with what was stated by (Nafiseh et al., 2019) that the use of magnesium oxide Nanoparticles led to a significant increase in hemoglobin parameters, red blood cell count (RBCs), packed cell count (PCV) and platelet count. The mechanism of the effect of magnesium oxide Nanoparticles on red blood

cell parameters is due to the formation of these cells within the stem cells in the bone marrow and any increase in their levels indicates that the size of the Nanoparticles (10-15 nm) and that the Nanoparticles have the ability to trigger an inflammatory response, which means an increase in red blood cell levels (Hauck et al., 2010).

The reason for the low level of hemoglobin in the blood may be due to a decrease in the rate of hemoglobin synthesis or an increase in the rate of its oxidation. This synthesis requires iron, which is provided from food sources and ferritin storage. Iron deficiency in laboratory animals is due to a deficiency or imbalance in food intake and thus a deficiency in the supply of iron, which is considered very important in the synthesis of hemoglobin (Mohamed et al., 2018).

(Cora et al., 2020) confirmed that exposure of male rats to bisphenol causes impairment of the neuroendocrine mechanism, which plays an important role in regulating food intake, leading to metabolic disorders. The decrease in red blood cell parameters is due to hemolysis of blood cells as a result of poisoning, which is responsible for the decrease in the hemoglobin level in the blood (Speath, 2008).

(Olson et al., 2000) explained when analyzing blood toxicity that any increase or decrease in blood levels indicates inhibition or increase in blood cells or that there is an imbalance between the destruction of blood cells and their production. Red blood cells are particularly sensitive to oxidative damage due to the large abundance of polyunsaturated fatty acids in their membranes as well as the high cellular concentration of oxygen and blood hemoglobin (Cappuzzo, 2009).

Effect of oral administration of MgO NPs on the number of white blood cells in rats:

The effect of oral administration of MgO NPs on the number of total white blood cells is shown in Table 3. The results showed a significant increase ($P \leq 0.05$) in the number of white blood cells in the rat groups treated with bisphenol M2, which reached 8.84 (×103/mm3) compared to the healthy control group M1, which was 6.43 (×103/mm3), and a significant decrease in the percentage of both lymphocytes and monocytes, which were 23.01, 5.66% respectively, compared to the healthy control group M1, which was 37.22, 9.62% respectively.

While a significant decrease was observed in the above parameters when treated with Nano magnesium oxide at different concentrations in groups M3, M4, as the number of white blood cells was 5.87, 6.09 (×103/mm3) respectively, and a significant increase occurred in lymphocytes to be 29.73, 33.48%, and in the percentage of monocytes to be 8.55, 8.91% respectively, compared to the groups of rats treated with bisphenol M2.

Table 3: Effect of oral administration of MgO NPs on the number of white blood cells in rats after 28 days of care.

Treatments	WBCs($\times 10^3/mm^3$)	Lymphocytes%	Monocytes%
M1	b 0.04±6.43	a 0.06±37.22	a 0.05±9.62
M2	a 0.06±8.84	e 0.02±23.01	c 0.5±5.66
M3	c 0.05 ±5.87	c 0.6±29.73	b 0.6±8.55
M4	b 0.05±6.09	ab 0.06±33.48	ab 0.23±8.91

Different letters in the same column indicate to significant differences at ($P \leq 0.05$).

The significant decrease in the percentage of lymphocytes exposed to bisphenol may be due to the increased rate of damage to the DNA of these cells as a result of excessive production of free radicals associated with oxidative stress (Rabia et al., 2020). The increase in the number of white blood cells in laboratory animals exposed to bisphenol can be explained by the role of bisphenol in activating inflammatory conditions and also as a result of stimulating the immune system and stress resulting from bisphenol (Alabi et al., 2021).

The increase in the levels of white blood cells is evidence of an inflammatory response, usually as a result of infection, and sometimes occurs due to the activation of the defense mechanism and the immune system against

the toxic effect, as it leads to an increase in the number of white blood cells as a result of any infection or any form of cytotoxicity or bone tumors (Lonare et al., 2014). These results are consistent with what was stated by (Nafiseh et al., 2019) that the use of magnesium oxide Nanoparticles led to a significant decrease in the number of white blood cells and a significant increase in lymphocytes and the percentage of monocytes.

Effect of oral administration of MgO NPs on immunoglobulin levels in rats:

Table 4 shows the effect of oral administration of Nano magnesium oxide MgO NPs on the values of immunoglobulins IgA, IgE and IgG for groups of rats treated with bisphenol for 28 days. The results showed a significant decrease ($P \leq 0.05$) in the values of immunoglobulin standards for groups of rats treated with bisphenol, which reached 59.45, 4.61, 389.71 mg/dL, respectively, compared to the healthy control group, which was at 279.92, 22.01, 501.07 mg/dL, respectively. The results also showed a significant increase in the values of immunoglobulin standards when adding Nano magnesium oxide at the above concentrations compared to the groups of rats treated with bisphenol M2.

Table 4: Effect of oral administration of MgO NPs on the values of IgG, IgA, and IgE (mg/dL) for rats after 28 days of care.

Treatments	IgA	IgE	IgG
M1	a 0.05 ± 279.92	b 0.5±22.01	a 0.05±501.07
M2	d 0.05 ± 59.45	d 0.04 ±4.61	d 0.05±389.71
M3	c 0.06 ±179.35	c 0.06±18.26	c 0.05 ±453.01
M4	b 0.05±199.02	a 0.05±29.09	b 0.04 ±492.04

Different letters in the same column indicate to significant differences at ($P \leq 0.05$).

Bisphenol causes many health problems such as decreased sex-specific neurodevelopment, uterine cancer, weakened immunity, and interference with cellular pathways. Bisphenol has an effect on laboratory animals and humans and causes health problems such as infertility, weight gain, behavioral changes, early puberty, prostate cancer, breast cancer, cardiovascular effects, obesity, and diabetes (Schug et al., 2012). It has been found that Nanocomposites have an effective effect in raising the level of immunoglobulins in laboratory animals, as these globulins are proteins that play a role in increasing immunity in the body against pathogenic types of fungi, bacteria and foreign substances entering it, as the IgA type is found in high concentrations in the mucous membranes, especially the digestive system and the lining of the respiratory tract, saliva and tears, and are considered the most widespread antibodies in the body. The IgG type is the most common type of antibodies, making up about 80% of antibodies. It is found in body fluids and its function is to protect the body from bacterial and viral infections. It also plays a role in inhibiting the effect of toxins, neutralizing them, and expelling them from the body. The IgE type is found in the skin and lungs, as well as in the mucous membranes. It is primarily associated with cases of allergies and is largely produced by the immune system with the presence of environmental antigens (AL-Jameel, 2022).

5. Conclusion

This study has proven that magnesium oxide Nanoparticles (MgO NPs) at different concentrations have the ability to reduce the toxicity caused by bisphenol A and reduce its effect on the relative weight of some internal organs, red blood cell parameters, white blood cell counts, and immunoglobulin values in male albino rats, as the use of MgO NPs led to these rates reaching close to their normal rates.

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