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A Study of the Protective Efficacy of Spirulina Algae Powder Against Biochemical Changes in Rats Treated with Cadmium Chloride

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P A P E R I N F O

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A B S T R A C T

The current study investigates ithe protective role of *Spirulina platensis* algae powder against oxidative stress caused by exposure to cadmium chloride in laboratory white rats Rattus norvegicus. The study was conducted from May (2023) to April (2024) at the University of Karbala / College of Education for Pure Sciences / Department of Biology. In this experiment (18) rats, Weighed (190-230g) with Age range of Fourteen weeks were divided systematically into three groups (6 for each). The first group was considered a negative control, where water and fodder were given freely. The second group (G2) was considered a positive control, treated withan aqueous solution of cadmium chloride at a dose of 1 mg/kg of body weight. The third group (G3) was considered a protective group, where Spirulina algae powder was given in does of 177 mg/kg. After three hours, it was given Cadmium chloride solution. At the end of the experimental period (30 days), the animals were anesthetized with chloroform in the closed method, then take blood from their heart and then stored until blood and biochemical parameter were performed.

The results of the current study showed that oral administration of cadmium chloride solution for 30 days to group (G2) led to a morale boost at the level ($P < 0.05$) in the liver enzymes level (ALP, ALT, (AST) as well as urea, creatinine, and blood electrolytes (K.Ca, Na). A decrease in the level (GSH), (CAT) and (SOD) was observed when compared to the (G1). As for the preventive role, the results showed that dosing the animals with Spirulina algae powder for a period of three hours before giving the animals the aqueous solution of cadmium chloride contributed to changing the aforementioned results by reducing the toxic effect of the cadmium chloride solution, as a significant decrease was observed at the level ($P < 0.05$) in the Liver enzymes level and kidney parameters when compared with (G2), and (G1). Antioxidant enzymes (GSH, SOD, CAT) increased in the above groups.

1. INTRODUCTION

The medicinal plants have occupied a large place in the medical sciences and pharmacy, have become a Safe basis for pharmaceutical materials of the current century. Recent studies have indicated the effect of medicinal plants as antioxidants as an alternative to drugs and chemical treatments. Modern scientific researches have also proven the pharmaceutical effectiveness of many compounds derived from plant sources that have antioxidant properties. This has made them occupy a large place in the field of medical treatment for many diseases that affect the physiological activity of humans and animals alike [1].

Spirulina algae is one of the plants that has been used in the treatment of many diseases. It has a positive effect on the body as it has received widespread attention from many researchers and scientists in addition to containing high concentrations of proteins and vitamins, especially vitamin B12. More over, it contains minerals, amino acids, polyunsaturated fatty acids, and biologically active components, including phycocyanin, beta-carotene, and phenolic compounds, which provide anti-inflammatory activity and antioxidant efficacy [2].

On the other hand, heavy elements are considered among the most dangerous chemical compounds that all living organisms are exposed to in the environment, including cadmium chloride, as its density is five times greater than the density of water. This makes it a cause of harm to the body, especially the kidneys since it causes tissue damage by disrupting the function of the renal tubules and failure of the natural absorption process of materials, thus reducing the process of phosphate absorption that occurs in the renal tubules, and then kidney failure occurs, as the kidneys contribute

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to the excretion of toxic waste, in addition to its effect on the liver, which constitutes an important site in the body as it plays a role in the processes of Biotransformation [3].

The rate of cadmium transfer from the liver to the kidney depends on the rate of formation of the cadmium-metallothionein (MT-Cd) complex in the liver. When the complex reaches the renal tubules, it is analyzed by lysosomes, which contain digestive enzymes that release cadmium. This in turn leads to the production of renal metallothionein, causing it to accumulate in the kidney at higher levels than in the liver. Since its free period in the kidney is long, it may reach 3 months, compared to the half-life of the MT-Cd complex, which reaches (only 4-3 days), which makes it harmful to the kidney and causes tissue damage in the tissue kidney [4].

The continuous increase in the concentration of heavy elements in the environment from its various sources was accompanied by the occurrence of many diseases, which made it a source of concern for all living organisms, especially humans, which was accompanied by the use of many medications to get rid of the symptoms of these diseases. Over time, the use of these medications became a major cause of many health problems, which are classified as side effects of using these medications. This prompted many researchers to find alternative methods to reduce the use of these medications by using alternative treatments that do not cause harm to the body. Thus, the present study aims at: Studying the protective role of Spirulina algae powder in reducing the renal toxic effects resulting from exposure to high concentrations of cadmium chloride. This is achieved by examining the changes occurr in the tissue structure of the kidney as the center for ridding the body of toxic wastes to which exposes the body to harm.

2. MATERIALS AND METHODS

male white laboratory rats, *Rattus norvegicus*, weighed between 190-330 grams and were aged Fourteen (we). They were btained from the animal house of the College of Pharmacy - University of Karbala. The experiment lasted from September 22 to January 15 of the academic year 2024. The animals Placed in boxes with mesh lids to adapt rats. The floor of the cages was covered with sawdust, taking into account changing it from time to time to maintain its cleanliness. The animals were given water and fodder freely, under suitable ventilation conditions, at a temperature of (25°C) and natural lighting, and were left to adapt to the experimental conditions for two weeks.

2.1. Experiment Design

After the acclimatization period for the animals ended, they were classified into three groups, with (6) animals for each group, as follows:

1-The first group (G1): The animals of this group were considered negative control and were given water and feed freely

2-The second group (G2): The animals of this group were considered positive control as they were dosed daily with cadmium chloride at a concentration of 1 mg/kg for (30) days [5].

3-The Third group (G3): The animals of this group were a preventive group. The animals were dosed daily with cadmium chloride at a concentration of (1) gram/kg of body weight, and after three hours they were dosed with spirulina powder at a concentration of 177 mg/kg of body weight for a period of (30) days [6].

2.2. Collection Blood

At the end of the experiment, the rats were anesthetized using the closed method, which included placing the animals in a tightly sealed container, noting that a cotton ball containing chloroform was placed inside the container. After a few minutes, the animal was anesthetized. Then, blood get directly from heart using a sterile medical syringe with a capacity of (5) ml. Then, the blood in test tubes was put .After that, the tubes were transferred to the centrifuge at a speed of (3000) rpm for (15) minutes in order to obtain the serum, which was transferred to small, clean, dry, and marked Eppendorf tube plastic tubes. The serum was kept in the refrigerator until blood and biochemical tests were performed.

3. RESULTS AND DISCUSSION

3.1. Effect of treatment with Cadmium Chloride Solution on Liver Enzyme Levels (AST, ALP, ALT)

Table 1 an increase was noted at ($P<0.05$) in the concentration of liver parameter (ALT, AST, ALP) in (G2) when compared with the negative control group (G1).This is due to damage in the liver tissue of animals treated with cadmium chloride because of its toxicity to the body. Our results were consistent with the study of Kang [7] who noted a significant increase in liver parameter levels exposed to cadmium chloride directly or indirectly through the accumulation of toxins in the body for long periods, which leads more in the levels of these parameter such as AST, ALP, ALT as a result of tissue damage in the liver .

These results confirm the results of the researchers [8] Asagba & Eriyamremu in their study where the animals were given (10) mg of cadmium chloride / kg of body

weight for 16 weeks. They noticed a significant increase on concentration of liver enzymes AST, ALT and ALP compared to the (G1) that received water and fodder freely. They explained that the cadmium chloride solution caused atrophy of the liver cells in the treated animals as a result of the liver poisoning that caused the destruction of the tissue of the cells which make up the liver and the release of larger quantities of these enzymes. However, the study did not match the study Wielgus-Serafińska *et al*. (9) which showed a decrease in the activity of various enzymes in the liver of mice after exposure to cadmium chloride solution, indicating liver damage.

TABLE 1. Shows the Level of Liver Parameter on Study Groups

Tretments	Means ± stander err		
	ALP	ALT	AST
Negative control group G1))	133.66± 0.34 C	24.95± 0.66 D	91.00± 0.19 C
Positive control group (G2)	176.48 ±1.70 A	44.14± 2.29 A	226.51± 9.33 A
Protective group (G3)	139.69 ±0.27 B	31.95± 0.64 C	118.17± 2.41 B
LSD	2.607	3.7406	14.223
P(value)	0.05	0.05	0.05

3.2. Effect of treatment with spirulina and cadmium chloride on the concentration of liver enzymes (AST, ALP, ALT)

The study showed in Table (1) that there was a more difference At (P<0.05) in enzyme parameters (ALT, AST, ALP) when comparing the (G2) with (G3) .The level of these parameter decreased in the (G3) group but the concentration these enzymes would l not decrease to return to normal concentrations in the (G1) group.

The study agreed with study conducted by Ibrahim et al [10] .It showed that animals treated with spirulina and cadmium chloride had a decrease in the level of liver parameter compared to those (G2), as spirulina powder contributed significantly to reducing the concentration of parameter ALT, ALP and AST by reducing the oxidative stress caused by the cadmium chloride solution on the body It contributes to the destruction of liver tissue and the release of free radicals that can destroy the roots of liver cells and thus release high concentrations of the enzyme in the blood. The study differed from the outcome of other studies conducted by researchers Damessa et al. [11], as liver enzymes did not show any decrease in animals treated with spirulina and cadmium chloride solution compared with animals treated with cadmium alone. This is related to the destruction of liver tissue and the inability of spirulina powder to rebuild the tissue, especially after a long period of treatment with cadmium.

3.3.The Effect of Treatment with Spirulina and Cadmium Chloride on the Concentration of Oxidation Enzymes (MDA, SOD, CAT, GSH)

The study in Table 2, showed a significant increase at (P<0.05) in level of antioxidant parameter (SOD, CAT, GSH)in the (G3) when compared with the (G2), accompanied by a non-significant decrease in the concentration (MDA) in the preventive group when compared with the positive control group, noting that these concentrations returned as close as possible to the normal state in the negative control group.

TABLE 2 . Shows level of antioxidant parameter in the study groups

Treatments	Means ± stander err			
	MDA	CAT	SOD	GSH
Negative control group G1)(	0.32±2 2.90 B	3.88±33.58 C	3.41±36.94 C	3.15±44.72 A
Positive control group (G2)	0.55±2 5.37 A	1.32±50.76 A	1.01±67.59 A	1.00±38.04 C
Protective group (G3)	0.36±2 1.51 C	3.94±40.17 B	3.02±59.49 B	3.54±43.30 AB
LSD	1.1291	7.5446	2.3907	2.6096
P(value)	0.05	0.05	0.05	0.05

The results of the current study were consistent with the results of the study by Candelaria et al. [12], where they observed an increase in the oxidation enzymes SOD, CAT, and GSH in the group fed on spirulina, accompanied by a decrease in the level of the MDA enzyme, as spirulina, which contains pigments, carotenoids, xanthophyll, vitamins, minerals, and other nutrients, caused a decrease in MDA and reduced the severity of cadmium toxicity within the body tissues .

In the same direction, the study agreed with Ibrahim et al.'s study [10], which showed an increase in the level of oxidation enzymes in the (G3) fed on spirulina and cadmium chloride, and that this increase came as a result of the role of spirulina in maintaining the body's tissues. This is because it contains important elements, including vitamins with high nutritional value, which help the body to curb the toxic effects resulting from exposure to cadmium chloride solution. At the same time, they contribute to raising the concentration of antioxidant enzymes and also reducing the concentration of the MDA enzyme.

3.4. The Treatment with Cadmium Chloride Influence on the Level of Kidney Standards (Na, Ca, K, urea, crea)

The study showed in Table (3), a significant increase at the (P<0.05) in the level of kidney standards (Na, Ca, K, urea, crea) in group (G2) compared with G1. The study was consistent with the results of Ogham etal. [13] who noted a significant increase at (P<0.05) in the standards of kidney parameters (creatinine, urea, K, CA, Na) for animals fed cadmium chloride compared to the normal group, as exposure of the body to high concentrations of cadmium chloride solution causes damage to kidney tissue and thus kidney failure.

Moreover, the study by Karami et al. [14] indicated that cadmium chloride caused an increase in kidney parameters, including (Na, Ca, K, urea, crea), which caused kidney dysfunction by delaying absorption and kidney tissue damage.

In the same direction, the study agreed with the study of Yan & Allen [15], where they observed an increase in the level of (Na, Ca, K, urea, crea) in animal models exposed to cadmium as a result of damage to some components of the renal unit, as cadmium causes a general defect in the epithelial cells lining the proximal convoluted tubule. This causes an increase in the secretion of renal electrolytes into the blood. The results of the current study differed from the study by Cui et al. [16], where they observed a decrease in the concentration of renal parameters, including Ca, K, and Na, as cadmium chloride caused damage to kidney tissue, represented by atrophy of the renal glomerulus and destruction of the cells lining the renal tubules, accompanied by damage to the mitochondrial membranes.

3.5. The effect of Treatment with Spirulina and Cadmium Chloride on the Concentration Of Kidney Parameters (Na, Ca, K, urea, crea)

The study Table 3 showed a significant drop at the level (P<0.05) in the concentration of kidney parameters (Na, Ca, K, urea, crea) in the preventive group (G3) that was given spirulina powder and cadmium chloride solution when compared with the positive control group (G2) that was given cadmium chloride solution only.

The results of the current study agreed with the results of the study of Ibrahim et al. [10] who observed a decrease in the level of kidney parameters in the group dosed with a solution on cadmium chloride and spirulina for 28 days and interpreted this as spirulina reducing the toxic effect of cadmium chloride as it provides lines of defense for the body and limits damage to kidney tissue. The results of the current study confirm the results of the study by Berbesh et al. [17] who used Spirulina platensis algae against toxicity caused by giving cadmium chloride solution to chickens. 45 chickens were randomly divided into three equal groups, 15 chickens for each group. The first group received water samples and several negative control groups, while the second group received a dose of cadmium chloride solution and was considered a positive control group, while the third group received a dose of cadmium chloride solution and spirulina and was considered a preventive group. The study showed that chickens dosed of cadmium chloride solution decreased significantly in weight, in addition to the deaths of many members of the group, while the members of the group that received a dose of cadmium chloride solution in addition to spirulina powder achieved a significant improvement in growth parameters, which included an improvement in final body weight and daily weight gain, in addition to a significant decrease in kidney physiological parameters. In the same direction, the results of the current study agreed with the results of the study of Gaurav et al. [18] who used spirulina and cadmium chloride, where the effect of giving cadmium chloride solution to mice for 30 days was studied. The experimental animals received cadmium chloride solution at a level of 2 mg/kg of body weight, while the animals of the second group were treated by giving them cadmium chloride solution simultaneously with spirulina powder for 30 days. It was observed that kidney function was restored to normal levels in the second group compared with mice exposed to cadmium chloride in the first group.

TABLE 3. Shows The Standard of Kidney Function in The Study Groups

Treatments	Means ± stander err Urea	Creatinin	Na	K	ca
Negative control group G1)(	0.29± 31.45 B	0.04 ± 0.82 B	1.30± 165.33 B	0.08± 3.38 B	0.13± 11.48 B
Positive control group (G2)	0.39± 41.31 A	0.15± 1.52 A	3.90± 185.67 A	0.13±2.4 6 C	0.35±1 2.88 A
Protective group (G3)	0.39 ±29.95 C	0.01±0.9 4 B	0.65 ± 163.17 B	0.15 ±3.43 B	0.29±1 0.33 D
LSD	0.9824	0.2377	6.4266	0.3617	0.7412
P(value)	0.05	0.05	0.05	0.05	0.05

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Arabic Abstract

تهدف الدراسة الحالية الى معرفة الدور الوقائي لمسحوق طحالب السبيرولينا بلاتينسز ضد الجهد التأكسدي الناتج عن التعرض لكلوريد الكادميوم في الجرذان المختبرية البيضاء. اجريت هذه الدراسة خلال شهر مايو 2023 الى شهر ابريل 2024 في جامعة كربلاء \ كلية التربية للعلوم الصرفة \ قسم علوم الحياة.

في هذه التجربة، 18 جرذ تتراوح اوزانهم بين (190- 230) غرام بمعدل عمر اربعه عشر اسبوع . قسمت نظاميا الى ثلاث مجاميع (ستة في كل مجموعه). حيث تعتبر المجموعة الاولى هي مجموعه السيطرة السالبة و التي اعطيت الماء و العلف فقط. المجموعة الثانية والتي تعتبر مجموعة سيطرة موجبة والتي عوملت بالمحلول المائي لكلوريد الكادميوم بجرعة و التي عوملت بالمحلول المائي لكلوريد الكادميوم بجرعة 1 غم\كغم من وزن الجسم. اما المجموعة الثالثة والتي تعتبر المجموعة الوقائية حيث عوملت بمسحوق طحالب السبيرولينا و بعد ثلاث ساعات اعطيت محلول كلوريد الكادميوم. بعد انتهاء مدة التجربة (30 يوماً) تم تخدير الحيوانات بمادة الكلوروفورم بطريقة مغلقة، بعدها تم جمع الدم و خزنت لحين اتمام التحاليل البايوكيميائية.

اشارت نتائج الدراسة الحالية الى ان التجريع الفموي لمحلول كلوريد الكادميوم لمدة 30 يوماً للمجموعة الثانية ادى الى زيادة معنوية عند مستوى ($P > 0.01$) في مستوى انزيمات الكبد (AST, ALT, ALP) و كذلك اليوريا , الكرياتنين و الكتروليات الدم .(K ,Ca, Na) , بينما ظهر انخفاض في مستويات (GSH) و (CAT) و (SOD) عند مقارنتها بالمجموعة الاولى .

كدور وقائي اظهرت النتائج ان تجريع الحيوانات بمسحوق طحالب السبيرولينا لمدة ثلاث ساعات قبل اعطاء المحلول المائي لكلوريد الكادميوم ساهم في تغيير النتائج المذكورة عن طريق تقليل التأثير السمي لمحلول كلوريد الكادميوم، حيث لوحظ انخفاض ملحوظ عند مستوى ($P > 0.05$) في مستويات انزيمات الكبد و معايير الكلى عندما تم مقارنتها مع المجموعة الثانية و الثالثة، وارتفعت كذلك الانزيمات المضادة للأكسدة (GSH, SOD, CAT) في المجموعة المذكورة.