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THE ROLE OF THE NANO EXTRACT OF *SPIRULINA PLATENSIS* IN IMPROVING THE NEGATIVE EFFECTS ON THE REPRODUCTIVE SYSTEM AND SOME PHYSIOLOGICAL PARAMETERS IN FEMALE RATS EXPOSED TO OXIDATIVE STRESS

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Abstract

This study was conducted to identify the role of the alcoholic extract of *Spirulina* algae nano-loaded with nano-zinc oxide in improving the negative effects of hydrogen peroxide-induced oxidative stress on the female reproductive system in rats. (35) female rats, whose weights ranged between (180-200) g, and their ages ranged between (8-9) weeks, were used in the experiment. The animals were randomly divided into seven groups, each group included five rats. The groups were divided in the experiment as follows: the control group was dosed with normal saline, the first treatment (T1) was treated with hydrogen peroxide in drinking water, and the second treatment (T2) was treated with alcoholic extract of *Spirulina platensis* at a concentration (300 mg/kg), the third treatment (T3) was treated with nano extract of *Spirulina platensis* at a concentration (250 mg/kg), the fourth treatment (T4) was treated with nano zinc oxide at a concentration (20 mg/l), the fifth treatment (T5) was treated with hydrogen peroxide and dosed with normal extract of *Spirulina platensis*, the sixth treatment (T6) was treated with hydrogen peroxide and dosed with nano extract of *Spirulina platensis*. After the end of the experiment, which lasted 21 days, the animals were anesthetized and their weights recorded, and blood was drawn from the heart directly for biochemical tests. The following organs (uterus and ovaries) were removed and their weights were recorded.

The results showed a significant decrease ($P<0.05$) in the first treatments (T1), the fifth (T5), and sixth (T6) in the average weight percentage of the uterus and ovaries compared with the control, while the second (T2), third (T3) and fourth (T4) treatments showed a decrease ($P>0.05$), but it did not reach the level of significance in the ovaries and the third treatment in uterus compound with control. The results showed a significant decrease ($P<0.05$) in the mean concentration of estrogen and follicle-stimulating hormone in the first treatment (T1), the fifth (T5) and the sixth (T6), The fourth treatment (T4) showed a significant decrease (T4) of progesterone concentration, and the second (T2), third (T3) and fourth (T4) treatments showed an insignificant increase ($P>0.05$)) in the concentration of hormones, except for the third treatment (T3) which witnessed a significant increase ($P<0.05$) in the concentration of the luteinizing hormone ,While the fifth (T5) and sixth (T6) treatments showed a non-significant ($P>0.05$) increase in the concentration of luteinizing hormone and progesterone, and the results showed a significant increase ($P<0.05$) in the concentration of urea and creatinine, while the increase was not significant ($P>0.05$) in the concentration of uric acid in the first treatment (T1) compared with the control and the rest of the treatments, while the second (T2) and the third (T3), the fourth (T4), the fifth (T5) and the sixth (T6) treatments showed a similarity in their rates, but they did not reach The level of significance when compared with the control in the concentration of creatinine, urea, and uric acid except for the third (T3) and the fifth (T5)

treatments which showed a significant decrease (P<0.05) in the concentration of uric acid lion compared with the control.

Keywords: Spirulina platensis, nanoparticles, peroxide hydrogen, reproductive system

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Introduction

Blue-green algae, to which the alga *Spirulina* belongs, are filamentous multicellular microorganisms with a bluish-green color characterized by their filamentous shape. Blue phycocyanin is the main pigment in photosynthesis (Ahsan *et al*., 2008), in addition to containing carotenoids and chlorophyll. It contains phycoarthanine, which gives it its red color and is also characterized by its high content of protein, essential amino acids, vitamins, and minerals (Ghaeni & Roomiani, 2016; Rohmah et al.,2022). It is found in tropical and subtropical alkaline lakes with high salinity. It is used to overcome malnutrition and improve body functions. It also has antioxidant properties, and it works to boost the immune system (Gurlek *et al*., 2019; Abbas et al.,2022). The algal extract also has anti-inflammatory properties and various pharmacological properties and works to prevent cataracts and allergic rhinitis (Abdella, 2010). Spirulina is also a food supplement that is very rich in iron. It is used in the fight against iron deficiency anemia. It also helps to relax blood vessels and is an anti-disease Liver (Mazokopakis *et al.,* 2014).

Nanotechnology is an important technology in many fields as it depends on synthesizing particles with nanoscale dimensions if these particles have different properties from the minerals that compose them (Visweswara Rao & Hua Gan, 2015). In recent years, interest in the production of nanoscale metal materials has increased. Because of their uses in a variety of fields in the fields of industrial, medical, and environmental vitality, where the importance of nanomaterials is due primarily to the high ratio of their surface to their size due to their extremely small and this feature increases the surface they exercise with other bodies (Gahlawat *et al*., 2016).

Oxidative stress is defined as an imbalance between the concentration of free radicals and the antioxidant defense systems in the body accompanied by an increase in lipid peroxidation leading to the breakdown of polyunsaturated fatty acids, resulting in damage to various body tissues (Pisoschi & Pop, 2015; Arif et al.,2023; Lafta et al.,2023; Margiana et al.,2022). Antioxidant includes enzymes and vitamins that work directly or indirectly to protect the body from the destructive effects of free radicals. Blood plasma is a good content of protective antioxidants and inhibits the processes of high lipid peroxides to avoid oxidative stress of protein components in the blood. There is a group of enzymes that have a role in removing the toxins of the active classes of oxygen, such as the enzyme superoxide dismutase and glutathione peroxidase, while the second group includes indirectly some enzymes to restore the internal antioxidants to their normal position (Ali *et al.,* 2020; Al-Jassani et al.,2022; Hussien et al.,2022).

Materials and working methods

Experimental animals

The study was conducted in the animal house affiliated with the College of the Education / University of Al-Qadisiyah, (35) female rats were used in the experiment, their ages ranged between (8-9) weeks, and their weights ranged between (180-200) g. They were placed in an air-conditioned room at a temperature of (23-25) C, and the light cycle (12 hours of light and 12 hours of darkness) per day and the animals were given free feeding and water for the duration of the experiment.

Experiment design

The animals used in the adult experiment (35) animals were divided into seven groups, each group included five animals, as follows:

1-Control: the group of animals that were dosed with the physiological solution for the duration of the experiment of 21 days.

2-Treatment: (T1) a group of animals that were dosed with hydrogen peroxide at a concentration of 1% through special vials for the duration of the experiment of 21 days

3-Treatment: (T2) a group of animals that were dosed with nano zinc oxide at a dose of 20 mg/kg of body weight for the duration of the experiment of 21 days.

4-Treatment: (T3) the group of animals that were dosed with the normal extract of algae at a dose of (300 mg/kg) of body weight for the duration of the experiment of 21 days.

5-Treatment: (T4) a group of animals that were dosed with algae nano extract at a dose of 250 mg/kg body weight for the duration of the experiment of 21 days.

6-Treatment: (T5) the group of animals that were dosed with hydrogen peroxide and the normal extract of algae at a dose of 300 mg/kg of body weight for the duration of the experiment of 21 days.

7-Treatment: (T6) a group of animals was dosed with hydrogen peroxide and algae nano extract at a dose of 250 mg/kg of body weight for the duration of the experiment of 21 days.

Moss collection and classification

Samples of *Spirulina platensis* were collected from different environments in Al-Qadisiyah Governorate and were classified by the specialist in the Department of the Biology / University of Al-Qadisiyah.

Preparation of the alcoholic extract of *Spirulina platensis*

The extraction was carried out in the Molecular Research Laboratory of the College of the Education / University of Al-Qadisiyah. After grinding the algae with an electric mill and turning it into a fine powder, the extraction process was carried out according to the method (Gebrehiwot *et al.,* 2019). Then the alcoholic extract of *spirulina* was loaded onto the nanomaterial according to the method (Karkos *et al.*, 2011) and after completing the loading process and with the required steps, the algae was placed in a plastic box to be used in the dosing and nano tests.

The daily oral dose of nano-zinc oxide was selected as 20 mg/L according to the study (Mason *et al.*, 2012) with the weekly dose. The dose of *Spirulina* algae extract was also selected based on previous studies that proved that the dose is 300 mg/kg of body weight. And the dose used for the nano-extract was 250 mg/kg of body weight, according to the study (Simsek *et al.*, 2009), and the experiment lasted 21 days.

Organ/body weight ratio

After completing the collection of blood samples, the rats were dissected and their organs (ovaries, uterus) were removed. The ratio of organ weight (gm) to body weight

(gm) was calculated according to the following equation:

$$\text{Percentage of the organ weight} = \frac{\text{organ Weight (gm)}}{\text{body weight (gm)}} \times 100$$

Then the organs were preserved with formalin (10%) for histological studies

Hormonal Parameters

The level of the hormones FSH, LH, Estrone, and Progesterone in the blood serum was measured using the Minividas device manufactured by the French company Biomerieux with several ready-made analyzes following the instructions attached with the examination kit for these hormones. Kidney function tests.

Measurement of serum creatinine

The serum creatinine level is measured according to the method (Islam *et al.*, 2004).

Measurement of serum urea concentration

The level of urea in the serum is measured using the Enzymatic Method, and a ready-made kit from the French company BioMerieux is used for this purpose.

Determination of serum uric acid concentration

The concentration of uric acid in the blood serum was determined using the analysis kit of the company (Biolabo, France).

statistical analysis

After data collection and tabulation, the statistical analysis program SPSS V.25 was used. Where the data were statistically analyzed according to the one-way ANOVA test, and the means of the trial groups were compared when the differences between them were significant using the Least Significant Difference (LSD) test at the significance level of 0.05 (Daniel & Cross, 2018).

Results and discussion

The results of the statistical analysis shown in the table (1) showed a significant decrease ($P<0.05$) in the first treatment (T1) in the average weight ratio of the ovaries and uterus compared with the control, while the second (T2), the third (T3) and the fourth (T4) treatments showed rapprochement in their rates. But it will not reach the level of significance ($P>0.05$) in the average weight percentage of the ovary and the third treatment (T3) of the weight of the uterus compared with the control. The second treatment (T2) and the fourth (T4) showed a significant decrease ($P<0.05$) compared with the control in the weight of the uterus. The results showed a significant decrease ($P<0.05$) in the fifth (T5) and sixth (T6) treatments in the average weight ratio of the ovaries and uterus compared with the control, while there was no significant difference ($P>0.05$) when comparing each other and the first treatment (T1). Except for the fifth treatment (T5), the weight of the uterus showed a significant decrease ($P<0.05$)) compared with the first (which was exposed to oxidative stress).

The improvement in the two treatments (T5) and (T6) (which were subjected to oxidative stress and then dosed with normal and nano extract) in the average weight ratio of the ovaries and uterus is agreeing with the results of the study (Khatun *et al*., 2018) and the reason for this is that the defense system that is characterized by algae *Spirulina*, which led to the protection of uterine and ovarian tissues from damage, as it showed an improvement in tissue structure, as well as *spirulina* containing antioxidants that prevent lipid peroxidation resulting from hydroxyl radical (Bhat *et al.*, 2001), and the reason for the improvement is attributed to the high efficiency of the nanocarrier on Absorbing substances, which is characterized by its ability to bind and carry the active compounds of algae,

and this is due to its relatively large surface area (Kim et al., 2020).

The decrease was shown by (T1 treatment) in the weight of the uterus and ovaries is consistent with the study (Bashandy *et al.*, 2016), where the reason for this is due to the formation of reactive oxygen species ROS, which leads to tissue damage, as it causes the disintegration and fragility of the cell membrane through lipid oxidation. (Sengupta, 2011). As for the changes in the parameters (T2, T3, and T4), it agrees with the study (Abadjieva *et al*., 2018), where the decrease in ovarian weight may be due to a low concentration of the extract or to a period that may not be sufficient for the required changes, which lasted 21 days, or It may be due to the increase in the number of corpus luteum, as these bodies have a high percentage of the weight of the ovary, estimated at 90%, which is formed after ovulation from the remaining parts of the Graafian follicle (Teh *et al*., 2014).

Table (1): shows the effect of alcoholic extract and nanocomposite of alga *platensis Spirulina* on the weight ratio of ovaries and uterus in rats induced experimentally with oxidative stress by hydrogen peroxide

The average weight ratio of the uterus (g/100g of the body weight)	**Ovary weight ratio)g/100g of the body weight)**	**parameters / The group**
0.37±0.15 A	0.18±0.012 A	**C**
0.18±0.020 C	0.05±0.006 B	**T1**
0.21±0.021 B	0.168±0.001 A	**T2**
0.35±0.019 A	0.164±0.003 A	**T3**
0.22±0.019 B	0.168±0.003 A	**T4**
0.15±0.006 D	0.06±0.001 B	**T5**
0.19±0.005 C	0.07±0.006 B	**T6**
0.026	0.11	**LSD**

The numbers indicate the mean ± standard error

C: The control group was dosed with physiological saline for the duration of the experiment (21 days).

T1: The first treatment represents the group of rats in which oxidative stress was induced by being dosed with hydrogen peroxide.

T2: The second treatment represents the group of rats that were dosed with alcoholic

extract of *S. Platensis* for the duration of the experiment.

T3: The third treatment represents the group of rats that were dosed with the Nonalcoholic extract of *S. Platensis* for the length of the experiment.

T4: The third treatment represents the group of rats that were dosed with nano-zinc oxide for the duration of the experiment

T5: The fourth treatment represents the group of rats that were dosed with hydrogen peroxide and the normal extract *S. Platensis* for the duration of the experiment.

T6: The fifth treatment represents the group of rats that were dosed with hydrogen peroxide and nano-extract *S. Platensis* for the duration of the experiment

The different letters indicate that there are significant differences between the groups (P<0.05).

The Similar letters indicate that there are no significant differences between groups (P>0.05)

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The results of the statistical analysis shown in Table (2) showed a significant decrease (P<0.05) in the treatments T1, T5, and T6 in the concentration of estradiol hormone compared with the control. The results also witnessed convergence in estradiol in treatments (T3, T4, and T2), but they did not reach the level of significance (P>0.05) compared with the control, and no differences appeared when comparing each other, and the results showed a significant (P<0.05) decrease in the level of follicle-stimulating hormone (FSH) and LH in the first treatment (T1) compared with the control. The second (T2), third (T3) and fourth (T4) treatments showed convergence in their rates, but they did not reach the level of significance (P>0.05) in the concentration of FSH and LH compared with the control except for the second treatment (T2) of LH. showed a significant (P<0.05) increase compared with the control, while the fifth (T5) and sixth (T6) treatments showed a significant (P<0.05) decrease in the level of follicle stimulating hormone compared with the control and an insignificant increase (P>0.05) in the level of luteinizing hormone compared to With control, there were no significant differences between the treatments when they were compared to each other Some, as for the progesterone hormone, the first treatment (T1) and the fourth (T4) showed a significant (P<0.05) decrease compared with the control, while the second (T2), the fifth (T5) and the sixth (T6) treatments showed a significant (P<0.05) increase compared to The control except for the third treatment (T3) witnessed a significant increase (P<0.05) compared with the control and the first treatment (T1).

Of the results shown in Table (2), the significant (P<0.05) decrease in treatment T1) (which was subjected to oxidative stress) in the concentrations of all hormones is consistent with the results of the study (Ebrahim, 2020), where the reason for the decrease is attributed to the effect of oxidizing substances indirectly on the ovaries by changing the regulation of the pituitary-gonadal axis, or the reason for the decrease is due to a decrease in the rate of gonadotropin release or an insufficient response of the pituitary gland to the gonadotropin-releasing hormone (Ryu *et al*., 2019). Antioxidant defense activities are one of the reasons for inhibiting the gonadotropin hormone (El-Demerdash *et al*., 2009). The results also showed a significant (P<0.05) increase in the treatments (T2 T3) in progesterone concentration and an insignificant increase (P>0.05) in the concentration of follicle stimulating hormone and luteinizing hormone, which agreed with the study (Abdel-Aziem *et al.,* 2018).

The reason for this is due to the active components of *spirulina* extract, which contain flavonoids, beta-carotene, and chlorophyll. The reason is also due to the strong antioxidant activity of algae extract

because it contains vitamins and phenols that play a role in antioxidant activities (AbouGabal *et al*., 2015). As for the improvement in terms of the decrease in the two treatments T5 and T6 (which were subjected to oxidative stress and dosed with alcoholic and nano algae extract) in the concentration of hormones (E2, LH, and (FSH) agreed with what was found by (Khatun *et al*., 2018). The reason for this is that *spirulina* protects against the effects of It is believed that the antioxidants in *spirulina*, especially phycocyanin, complex vitamin B12, and chlorophyll, work in an effective way to restore the antioxidant status of the ovaries (Chamorro-Cevallos *et al.,* 2008) or explain the reason for this to the role of nanocarriers in increasing the therapeutic ability of the nano-extract through Increased bioavailability and biological efficacy while decreasing the effects of oxidative stress (Zheng *et al.,* 2013).

Table (2): shows the effect of the alcoholic and compound extract of Spirulina platensis on some hormonal parameters in rats induced experimentally with oxidative stress by hydrogen peroxide

Progesterone)ng/ml(	**LH unit/ml)(**	**FSH) unit/ml(**	**Estradiol (E2)) pg/ml(**	Standards the group
10.66±0.19 B	**0.163±0.017 B**	**0.193±0.006 A**	**499.95±39.32 A**	**C**
5.07±0.22 C	**0.103±0.003 C**	**0.110±0.01 C**	**202.05±1.17 C**	**T1**
16.19±0.64 A	**0.146±0.014 B**	**0.216±0.07 A**	**536.29±19.56 A**	**T2**
12.40±1.29 B	**0.210±0.005 A**	**0.200±0.02 A**	**550.02±14.39 A**	**T3**
6.44±0.87 C	**0.173±0.003 B**	**0.196±0.03 A**	**447.22±23.94 A**	**T4**
16.62±2.13 A	**0.166±0.013 B**	**0.173±0.02 B**	**254.84±12.20 C**	**T5**
16.99±1.65 A	**0.171±0.01 B**	**0.146±0.11 B**	**321.40±53.35 B**	**T6**
2.94	**0.03**	**0.033**	**69.45**	**LSD**

The numbers indicate the mean ± standard error

C: The control group was dosed with physiological saline for the duration of the experiment (21 days).

T1: The first treatment represents the group of rats in which oxidative stress was induced by being dosed with hydrogen peroxide.

T2: The second treatment represents the group of rats that were dosed with alcoholic extract of *S. Platensis* for the duration of the experiment.

T3: The third treatment represents the group of rats that were dosed with the Nonalcoholic extract of *S. Platensis* for the length of the experiment.

T4: The third treatment represents the group of rats that were dosed with nano-zinc oxide for the duration of the experiment

T5: The fourth treatment represents the group of rats that were dosed with hydrogen peroxide and the normal extract *S. Platensis* for the duration of the experiment.

T6: The fifth treatment represents the group of rats that were dosed with hydrogen peroxide and nano-extract *S. Platensis* for the duration of the experiment

The different letters indicate that there are significant differences between the groups ($P<0.05$).

The Similar letters indicate that there are no significant differences between groups ($P>0.05$)

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The results of the statistical analysis shown in Table (3) showed a significant increase in the concentration of urea and creatinine and an insignificant increase in the concentration of uric acid in the first treatment compared with the control and the rest of the treatments, while the second treatments (T2), third (T3) and fourth (T4) showed Convergence in its rates, but it did not reach the level of significance (in the concentration of urea, creatinine and uric acid except for treatment T2) of uric acid showed a significant decrease ($P<0.05$) compared with the control, and the results also showed a significant decrease in the concentration of urea, creatinine and uric acid in the fifth treatment (T5) and the sixth (T6)) (which was subjected to oxidative stress and dosed the normal and nano-extract) compared with the first treatment (T1) (which was subjected to oxidative stress)

The improvement in terms of the reduction in oxidative stress in the treatments in which nano-extracts (T6, T5) were used is due to the efficiency of the nano-extract and the increase in bioavailability. cells (Wang *et al.,* 2014; Hafsan et al.,2022). The results also showed a significant ($P<0.05$) increase in the concentration of urea and creatinine and an insignificantly ($P>0.05$) increase in the concentration of uric acid in the first treatment (T1), and this indicates damage to the liver and kidneys, and these results were consistent with (Bashandy *et al*., 2016; Huldani et al.,2022) or it may be due to a decrease in glomerular filtration and thus reduce the ability of the kidneys to excrete wastes (Andrew *et al.,* 2018; Zadeh et al.,2022).

Table (1-3) shows the effect of the alcoholic extract and the nanocomposite of *Spirulina Platensis* on some kidney functions in rats induced by oxidative stress experimentally by hydrogen

Parameters / Group	**urea)g/100ml(**	**creatinine)g/100ml(**	**uric acid)g/100ml(**
C	**39.50±4.57 B**	**0.36±0.08 B**	**2.13±0.47 AB**
T1	**48.14±2.47 A**	**0.72±0.08 A**	**2.53±0.33 A**
T2	**36.45±5.26 B**	**0.40±0.05 B**	**1.93±0.14 B**

T3	**37.45±0.76 B**	**0.36±0.08 B**	**1.20±0.11 C**
T4	**33.48±2.06 B**	**0.34±0.11 B**	**1.78±0.26 B**
T5	**35.60±3.66 B**	**0.50±0.11 B**	**1.10±0.17 C**
T6	**31.20±1.96 B**	**0.40±0.10 B**	**1.90±0.13 B**
LSD	**8.06**	**0.23**	**0.643**

The numbers indicate the mean ± standard error

C: The control group was dosed with physiological saline for the duration of the experiment (21 days).

T1: The first treatment represents the group of rats in which oxidative stress was induced by being dosed with hydrogen peroxide.

T2: The second treatment represents the group of rats that were dosed with alcoholic extract of *S. Platensis* for the duration of the experiment.

T3: The third treatment represents the group of rats that were dosed with the Nano alcoholic extract of *S. Platensis* for the length of the experiment.

T4: The third treatment represents the group of rats that were dosed with nano-zinc oxide for the duration of the experiment

T5: The fourth treatment represents the group of rats that were dosed with hydrogen peroxide and the normal extract S. *Platensis* for the duration of the experiment.

T6: The fifth treatment represents the group of rats that were dosed with hydrogen peroxide and nano-extract *S. Platensis* for the duration of the experiment

The different letters indicate that there are significant differences between the groups ($P<0.05$).

The Similar letters indicate that there are no significant differences between groups ($P>0.05$)

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Compliance with Ethical Standards statements

I. Ethical approval:

The manuscript is written in original and all the data, results pertaining to this manuscript are original according to the research performed. The authors followed academic integrity and have not copied any content/results from another source.

II. Funding details (In case of Funding):

The authors of this manuscript did not receive any funding to perform the present research

III. Conflict of interest

The authors of the study do not have any conflict of interest

IV. Informed Consent:

The authors of the manuscript agrees to publish this research in the journal if it's considerable by the editors of the journal. The authors provide full consent for reviewing and publishing this manuscript.

V. All the authors of this study contributed equally in terms of performing the research as well as in preparing the manuscript. All the authors of the study followed the guidelines of the corresponding author.

Any query/suggestion related to the manuscript can be reached to the corresponding author

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