Physiological effects of manganese nanoparticles on Alloxan-Induced Diabetes Mellitus in Adult Male Rats

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ABSTRACT

This study was conducted on the creation of the nanopolitan manganese particles and the study of the physiological effect of the nanopolitan magnes.The results or indicators of blood sugar showed the confirmation of low levels of sugar in the group of diabetes treatment by giving oral throughout the experiment period (28 days). In addition to the high percentage of insulin in the affected group and the treatment of Mn-NPs compared to the affected (positive) control group during the study period. The current study aimed to achieve preventive effects against diabetes. It turned out that eating the nanoparticles and within the permissible concentration 2 mg /kg per day reduces blood sugar and contributes to losing weight, as its consumption is related to a decrease in the risk of type 2 diabetes and reduces the level Blood Glucose in Adult Male Rats.

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التاثيرات الفيسيولوجية لجسيمات المنغنيز النانوية على داء السكري المستحدث بألالوكسان في ذكور الجرذان البالغة

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الخلاصة

أجريت هذه الدراسة على تخليق جسيمات المنغنيز النانوية ودراسة تاثيراتها الفيسيولوجية خلال فترة التجربة والبالغة (28 يومًا). بالإضافة إلى النسبة المئوية العالية من الأنسولين في المجموعة المصابة والمعالجة على جسيمات MN-NPs النانوية مقارنة بمجموعة السيطرة (الموجبة) خلال فترة التجربة. كما وهدفت الدراسة إلى تحقيق الآثار الوقائية ضد مرض السكري المستحدث من النوع الثاني. اوضحت النتائج أن تناول جسيمات MN-NPs النانوية وضمن التركيز المسموح به 2 ملغ /كغم يوميًا يقلل من نسبة السكر في الدم ويساهم في فقدان الوزن ، حيث يرتبط استهلاكه بانخفاض خطر الإصابة بمرض السكري وخفض مستوى الكلوكوز في دم ذكور الجرذان البالغة.

الكلمات المفتاحية: انزيمات الكبد, جسيمات المنغنيز النانوية, الالوكسان, مرض السكري المستحدث.

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INTRODUCTION

Nanotechnology, also known as nanomaterials technology, is a cutting-edge field of study because it bridges traditional scientific disciplines and has far-reaching practical implications. When compared to other sciences and technologies, this one's importance in meeting human needs stands out most clearly in the fields of chemistry, physics, materials science, molecular biology, and medicine (Sondi and Salopek, 2004). The study of nanomaterials, their characterization, the investigation of their physical, chemical, and mechanical properties, as well as the understanding of phenomena related to their small sizes, is what is known as nanoscience (Buzea *et al.,* 2007). Tissue engineering, cancer treatment, sports equipment, solar cells, space materials, and cosmetics are just some of the many fields that have benefited from the use of nanotechnology (Bachhav & Deore, 2015). The processes used to create nanoparticles are distinct from those used to create materials with particles larger than 100 nanometers in size because the particles or atoms that make up nanoparticles have different propertie (Gaffet, 2011). Their high surface-to-volume ratio (Mahendra *et al.,* 2009). and other physical and chemical properties, including as solubility, strength, diffusivity, toxicity, magnetism, optics, and thermodynamics, set them apart (Aswathy *et al.,* 2013).

Nanoparticles can be obtained through chemical, physical, or even biological means (Xi-Feng *et al.,* 2016). When it comes to issues of human health and the environment, the employment of chemical and physical procedures is not only prohibitively expensive, but also has negative consequences and necessitates the provision of unique conditions for chemicals, energy sources, pressure, and high temperature. As a result, efforts are directed toward developing biological methods for preparing these nanoparticles, since these have been shown to be not only simple to execute but also safe to use with health and environmental implications, not to mention cheap and environmentally friendly (Shakeel *et al.,* 2016). Fungi are one of the most important organisms utilized in the manufacturing of nanoparticles, and their ability to make metabolic chemicals like salts or chlorides from metallurgical components like gold, silver, zinc, and aluminum is crucial to the success of biological techniques of synthesis (Alaa, 2013). Aspergillus niger is characterized by its exceptional capacity for producing large numbers of conidia and by its capacity to produce many extracellular enzymes that play the primary role in analyzing the basic substance, converting it into its smaller components, and facilitating the fungi's absorption (Bensons, 2015). From the foregoing, the aim of this study was is the production of manganese nanoparticles Mn-NPs and the identification of particles and properties that distinguish them. The experimental development of diabetes mellitus in laboratory animals led to the advancement of science and knowledge, and work to find many reliable methods of treatment and knowledge of the main causes of the disease, and one of these methods is the experimental development of the disease in laboratory animals as alternatives to

humans. The first to develop this disease by surgically removing the pancreas in dogs, and some chemicals were used to cause diabetes, such as Alloxan ALX, and Streptozotocin STZ (Panneerselvam and Govindasamy, 2004).

MATERIALS AND METHODS

To get the biomass ready, we punched holes in the margins of the expanding pure fungal colony on the fungal growth plate with a sterile 6 mm cork punch and removed a disc from the center. To generate a fungal mat of varying densities, the disc was moved to a conical flask holding 100 ml of PGB liquid medium, where it was placed silently and gently to settle on the surface of the liquid medium before being deposited in the incubator at a temperature of 25-26 ºC for 5-7 days (Jayandran *et al.,* 2015).

The medium for the fungus was prepared and the medium was inoculated with the fungus, then it was transferred to the shaking incubator at 28 ºC for a week until the medium was completely consumed. After the fungal mat of *Aspergillus niger* was formed, the biomass was isolated by filtration, using multiple layers of medical gauze or sterile filter paper Whatman No: 1 Then the biomass is washed with deionized water 3-4 times until the remnants of the medium in the biomass are removed (a sign of this is that the water is clear and transparent from the filtration funnel). hen 10 g of biomass was weighed and crushed in a sterile ceramic mortar and placed in a conical flask containing 100 ml sterile deionized water and left for 24-72 hours in the shaking incubator, then the biomass was filtered again using filter paper, The biomass was disposed of, and the fungal filtrate was taken in an amount of 10 ml. It was mixed with 10 ml of manganese salt solution (0.4 mmolary) in a glass flask and incubated in a shaking incubator for 3 days in completely dark conditions until the color changed and a pale yellow color was obtained. Reduction of metal ions and indicates the complete stability of Mn NPs (Karbasian *et al.,* 2008).

Preparation of Mn NPs Nanoparticle Samples for Examination

Absorption UV-Visible Light Spectroscopy: The sample was prepared to examine the absorbance spectrum after 72 hours of placing the MnNPs solution in the incubator at 26-25 ºC in dark conditions, then 2 ml of the prepared MnNPs solution was taken after filtering it from mushrooms using sterile filter paper Whatman No1 and shaken well for homogeneity of the solution and examined by a Visible UV devicein the central laboratory of the University of Tikrit after filtering the device with sterile distilled water. UV-Vis spectrophotometry is considered one of the important techniques for detecting nanostructures (Jayaseelan *et al.,* 2012).

Measurement of the Particle Size of Nanoparticles: The sample was prepared in a suspended form containing nanoparticles, after which the sample was exposed to ultrasound waves to ensure that the particles were dispersed in a way that prevents agglomeration, then the cell of the device was washed with distilled water and dried well, Then, 4 ml of the filtrate was placed in it and placed in the device. The refractive index of the nanoparticles was fixed and the test was used to find out the size distribution of the particles inside the solution (Varenne *et al.,* 2016).

Biological Experiment

Experimental Animals

In this study, male white rats, aged 5-6 weeks and weighing between 175-180 g, were used in this study, which were obtained from the College of Veterinary Medicine / University of Tikrit, and placed in metal cages with metal covers and floors furnished with sawdust. Replace sawdust every two days. The animals were subjected to laboratory conditions of a photo cycle divided into 12 hours of light and 12 hours of darkness, and the temperature was fixed at 25 degrees Celsius. The animals were left for two days to adapt to the new conditions and to ensure that they are free from diseases. They were given food and water continuously (adlibitum) in sufficient quantities throughout the breeding period.

Induction of Experimental Diabetes

Experimental diabetes was induced in male rats by injecting them subcutaneously with Alloxan (British BDH company) according to the method mentioned in a study (Pradeep & Ashoka, 2015).

Preparing Weighted Food

Weighted food was prepared according to the National Academy of Science/National Research Council NAS/NRC 1978 to contain 12.5% casein from Saudi origin, 10% corn oil from Saudi origin, 5% cellulose, 10% sucrose from UAE, 1. 2% mixed minerals, 2% mixed vitamins, 59.30% Dutch starch. Distilled water was added to the mixture to make a cohesive dough and to form pieces suitable for feeding the rats, then they were placed in flat dishes made of stainless steel and It was dried in an oven at a temperature of 50 ° C by hot air until drying is complete, then it was packed in polyethylene bags and kept in a refrigerator at a temperature of 5 °C throughout the experiment period.

Experiment Design

Laboratory animals were obtained from the College of Veterinary Medicine / University of Tikrit. They are adult male rats, at the age of 5-6 weeks, and the weight ranges between 175-180 g. The experimental animals were randomly distributed into seven groups, each group consisting of 4 animals. Dosing was carried out orally with types of nano-manganese under study, according to the type of treatment, and the temperature was 20-25 degrees Celsius, and the lighting period was not less than 12 hours per day during the duration of the experiment, and the dose continued for 28 days.

The control group (negative control group): These animals were left uninjured and water and food were given above throughout the duration of the experiment.

The second group (positive control group): Diabetes was experimentally induced in this group and left without treatment, with water and food continued to be given throughout the duration of the experiment.

The third group: was fed the standard meal with 2 ml/kg of Mn-NP.
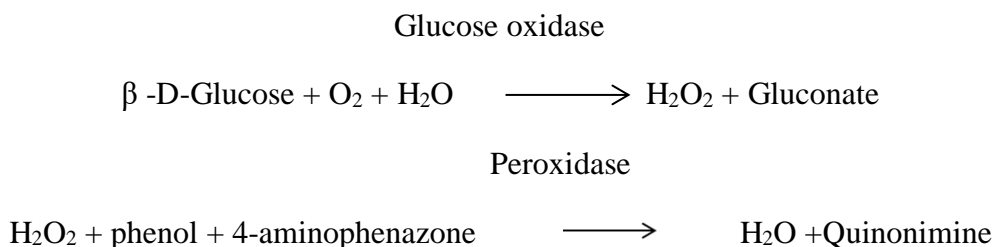
Blood Samples Collected

Immediately after the end of the experiment, the animals were starved for 10 hours and anesthetized with chloroform, by placing the animal inside an airtight glass container, and blood samples were collected by drawing blood from the heart using a syringe, where approximately 6-8 ml of blood was withdrawn and placed in plastic tubes that were centrifuged using Centrifuge at a

speed of 3000 rpm for 15 minutes to obtain serum that was kept at a temperature of -20 °C until analysis (Alarcon *et al.,* 1998).

Biochemical blood tests

Estimation of Glucose Concentration in the Blood Serum

The blood glucose concentration was measured using a glucose analysis kit (Kit) from the French company Biolabo. Glucose is estimated by an enzymatic method, as the glucose sugar is oxidized to Quinonimine dye, as shown in the reaction below:

Glucose oxidase

$$\beta\text{-D-Glucose} + O_2 + H_2O \longrightarrow H_2O_2 + Gluconate$$

Peroxidase

$$H_2O_2 + phenol + 4\text{-aminophenazone} \longrightarrow H_2O + Quinonimine$$

The color intensity of the complex resulting from the reaction was read using a spectrophotometer from the French company CECL at a wavelength of 500 nm, then the glucose concentration was calculated according to the following equation:

Blood Glucose Concentration (mg/100ml)=(sample absorbance)/(Absorbance of the standard solution)×standard Solution Concentration (100 mg/100 ml)

Estimating Blood Serum Insulin Concentration

The concentration of the insulin hormone was measured by following the steps provided with the ready-made analysis kit and according to the instructions of the American manufacturer (Monobind) for the enzyme linked immunosorbent assays ELISA technique (Tietiz, 2005).

Enzyme tests

The enzyme activity was estimated using ready-made kits from the company Roche (Germany). Reading the results using the Swiss Reflotron device according to the company's instructions and equipment as mentioned in (Tietiz, 2005).

RESULTS AND DISCUSSION

The biosynthesis of A. niger fungal filtrate was shown to be effective in transforming Mn-NPs' precursor chemicals into nanoparticles, as demonstrated by the results Fig. 1. Although the leachate from the fungus was colorless before being treated with the manganese solution, after treatment the leachate took on a faint yellow hue Figure 2AFigure 2B. The color change may have been caused by surface plasmon excitation (the basis of this vibration is for electron conduction groups), which is an early indicator of the ability of fungal metabolic products to break down the metal compounds added to them and form nanoparticles as a result of the reductive action of Mn-NPs (Vahid, 2018).
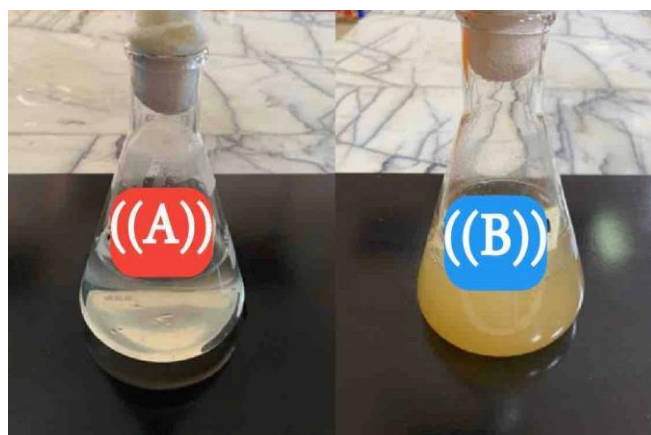
Figure 1. The change of color of manganese solution, A: fungus filtrate at the beginning of addition, B: manganese nanoparticles after 72 hours of incubation.

The peak of absorption in the case of manganese nanoparticles Mn-NPs was found to be at a wavelength of 421 nm, demonstrating that the fungus A. niger is capable of synthesizing manganese nanoparticles, which is the specific wavelength range for absorption X-rays from manganese nanoparticles, Fig. 2. The results agreed with what was found by (Sboui *et al.,* 2010). that the wavelength of manganese nanoparticles is within the range of 421 nm.
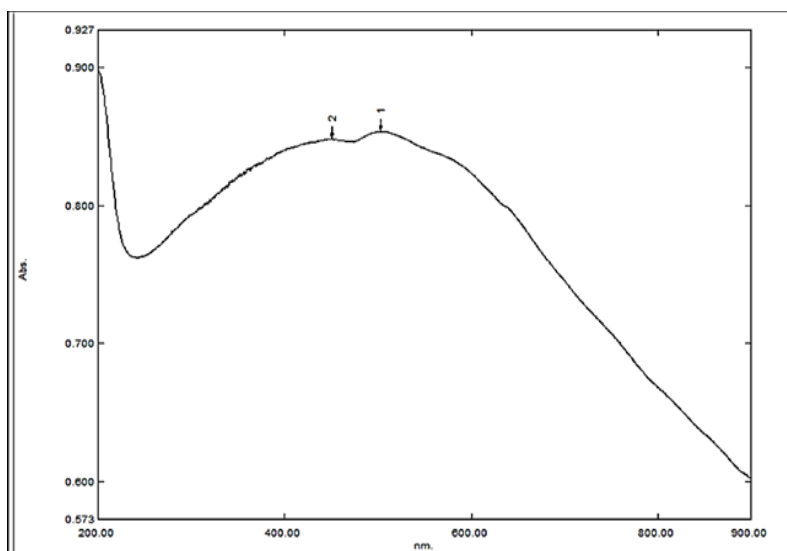

Figure 2. Shows the absorption peak in the case of Mn-NPs at the wavelength of 421 nm.

Distribution Size of Nanoparticles

Particle size measurement is one of the most common types of tests used to learn about nanoparticle attributes, since it provides insight into nanoparticle size and the distribution of nanoparticles in a solution in relation to density. The results of the particle size calculation showed that the size of the Mn-NPs was distributed in the suspended sample at a rate of 87.39 nm, which was proportional to its density inside the solution, Figure 3, Which agreed with what was found by (Shehata & Moussa, 2014). that the Mn-NPs were in the form of globules.
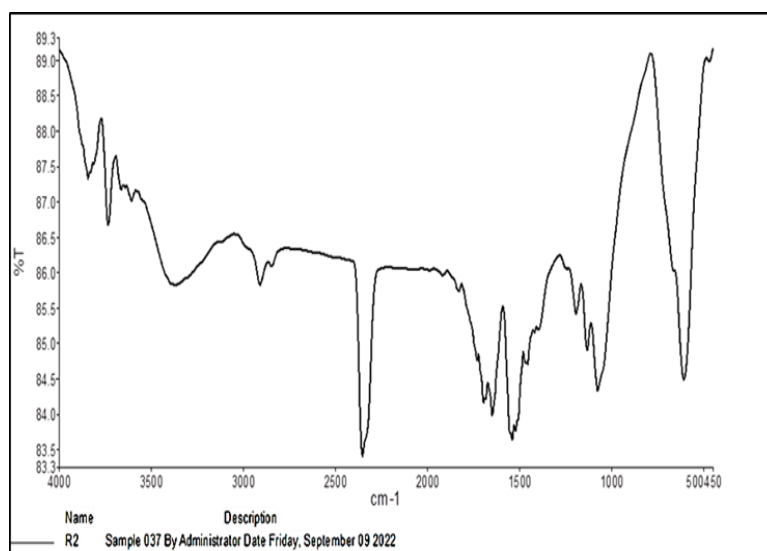
Figure 3. Volume distribution of Mn-NPs nanoparticles in the solution.

Effect on Glucose Concentration

The results of the current study showed in Table No. 1 that the induction of diabetes by alloxan led to a significant increase (P<0.05) in the concentration of glucose level in the blood serum of male rats with induced diabetes mellitus and untreated throughout the duration of the experiment compared with the healthy control group

The reason is that alloxan, which was given to mice, attacked B-cells. This leads to the production of large amounts of free radicals that accumulate and become toxic and destroy the pancreatic beta cells responsible for insulin production. It may also have caused the development of insulin resistance and disruption of the functions of cellular receptors for insulin, thus stopping the process of receiving cells for glucose and activating the processes of glycogenolysis and the formation of glucose from non-carbohydrate sources This result agreed with (Lowor & Amoah, 2008). in diabetic rabbits, (Toci *et al.*, 2009). in diabetic rats, and (Soh-Hyun *et al.,* 2013). in neodiabetic rats.

Table 1. The effect of Mn-NPs on the concentration of glucose (mg/100 ml) in the blood serum of healthy rats and those with newly diagnosed diabetes who were given orally for a period of 28 days

Group	After the injury	The first week	The second week	The third week	The fourth week end of the experiment
G1	4.008 ±1.00ab	85.50 ±1.50c	87.50 ±0.50c	88.50 ±0.50c	86.50 ±0.50c
G2	253.00 ±1.00a	255.00 ±1.00a	258.50 ±0.50a	261.50 ±0.50a	264.00 ±1.00a
G3	251.50±1.50ab	244.00±2.00cb	187.00±1.00d	169.00±1.00ed	161.00±2.00 d

The different letters on the rates in one column indicate that there are significant differences between the rates at a probability level of 0.05.

G1: healthy control animals group, G2 non-diabetic animals group, G3 Mn-NPs animals group.

The results or indicators of blood sugar showed confirmation of infection before starting treatment for the infected group of animals (positive control) and the third group of animals given Mn-NPs and they were at 253.0 and 251.8 mg/dl respectively when compared with the uninfected group of

negative control animals which were at 84.0 mg/dl. When estimating the glucose concentration in the first week of the experiment, the results showed a significant decrease in the group of animals given Mn-NPs and it was at 244.0mg/dl, respectively, compared to the blood sugar concentration in the group of infected animals, which was at 255.0 mg/dl. While in the second week of treatment, a significant decrease was also observed in the blood sugar concentration of the group of animals given Mn-NPs, and it was at 187.0 mg/dl, respectively, when compared with the infected group, which was at 258.5 mg/dl. In the third week, the blood sugar concentration also decreased significantly in the animals of the group given Mn-NPs, as the glucose concentration was at 169.0 mg/dl, respectively, when compared with the infected group animals, in which the glucose concentration was at 261.5 mg/dl. As well as the concentration of blood sugar in the fourth and last week of the experiment, there was a significant decrease in its concentration in the animals of the group Mn-NPs, which was at 161.0 mg/dl, respectively, when compared with the animals of the infected group, which was at 264.0 mg/dl. The glucose-lowering effect may be explained by the antioxidant activity of Mn-NPs (Patrik Nasr *et al.,* 2019). Numerous studies have shown that manganese lowers blood glucose levels by stimulating the pancreas to produce insulin, which the body needs to regulate blood sugar levels and boost the immune system (Takeda, 2003).

Table (3) shows that there is a significant increase ($p<0.05$) in the concentration of insulin hormone in the blood serum of experimental diabetic rats. It is noted from the table that after four weeks of oral feeding with (Mn-NPs), the insulin hormone level increased by 3.1%. Compared with the infected control group 1.0%.

Table 2. The effect of Mn-NPs on the insulin concentration (mg/100 ml) in the blood serum of healthy rats with induced diabetes, which were given orally for a period of 28 days.

Group	Insulin concentration
G1	c 0.50 ± 28.50
G2	b 1.00 ± 67.00
G3	a 2.00 ± 52.00

The different letters on the rates in one column indicate that there are significant differences between the rates at a probability level of 0.05.
G1: the group of healthy control animals, G2 the group of animals with induces diabetes without treatment, G3 the group treated with Mn-NPs.

These results are also consistent with what was mentioned by (Soh-Hyun et al., 2013). that Mn-NPs has an effect in balancing the level of glucose in people with diabetes, stimulating the work of the pancreas to produce insulin, and preventing further destruction of beta cells, in addition to some improvement in its function.

The results of Table 3 showed that there was a significant increase in the liver enzyme AST at the level of probability 0.05 among the affected control subjects compared to the healthy control subjects, as it was 5.20 and 1.50 U/L. As for the treatment with Mn-NPs, the concentration of liver enzyme AST recorded a significant decrease at 3.10 U/L. Followed by the results of the table also showed a significant increase at the level of probability 0.05 for the liver enzyme ALT among the affected control treatment compared to the healthy control treatment, as it recorded 4.10 and 1.00 U/L, respectively. As for the treatment with Mn-NPs, the concentration of the liver enzyme ALT recorded a significant decrease at 2.50 U/L. The results of the table also showed a significant increase at the level of probability 0.05 for the liver enzyme ALP among the infected untreated control subjects compared to the healthy control subjects. It recorded 6.00 and 4.50 U/L. As for the group treated with

Mn-NPs, the concentration of liver enzyme ALP recorded a significant decrease at 3.00 U/L, and this indicates the effectiveness of green coffee in reducing the concentration of liver enzymes. The results agreed with what was stated in the study (Patrik *et al.,* 2021). that the improvement in the level of enzymes is due to the fact that Mn-NPs act as a protective substance for the liver, and have an improved role against liver damage caused by oxidative stress, improving its vital functions and reducing the damage of oxidative stress It works to analyze free radicals and prevent the destruction of cell membranes through oxidation, thus preserving the values of enzymes(Takeda, 2003).

Table 3. The effect of Mn-NPs on the level of liver enzymes in the blood serum of healthy male rats with INDUCED diabetes and given orally for a period of 28 days

Group	AST U/L	ALT U/L	ALP U/L
G1	c 1.50 ± 58.50	c 1.00 ± 40.00	c 4.50 ± 127.50
G2	a 5.20 ± 87.00	a 4.10 ± 50.00	a 6.00 ± 188.00
G3	b 3.10 ± 75.50	b 2.50 ± 47.00	b 3.00 ± 179.00

The different letters on the rates in one column indicate that there are significant differences between the rates at a probability level of 0.05.

G1: the group of healthy control animals, G2 the group of animals with induced diabetes without treatment, G3 the group treated with Mn-NPs.

CONCLUSION

Eating manganese and within the permissible concentration of 2 mg /kg per day reduces Glucose blood and contributes to weight loss as its consumption is related to a decrease in the risk of the second type of diabetes and low -level Glucose blood and a decrease in the effectiveness of AST, Alt and Alp enzymes and the cause of this The decrease is due to the active ingredients found in the nanoparticles of manganese, which is as an antioxidant substance that analyzes free radicals and prevents the collapse of the cell membranes by oxidation and thus maintaining the values of enzymes.

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