



Moringa Oleifera L. Extract Modifies Oxidative Stress and Metabolic Changes Associated with Diabetes in Male Rats

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Abstract

Diabetes is a metabolic illness marked by chronically high blood sugar levels caused by oxidative stress and free radicals, which leads to pancreatic beta cell malfunction and insulin resistance, worsening the condition. This study investigated the protective effect of moringa oleifera leaf extract (MLE) on some biochemical variables in diabetic male rats treated with alloxan, which increased blood sugar levels. Male rats weighing 180 ± 150 g on average were placed in four groups, each containing five male rats. Several major biochemical tests were conducted on glucose, HbA1c, lipid levels, renal function indices, and antioxidant levels. The results showed that MLE significantly reduced glucose and HbA1c levels, lipid profile, and kidney function while enhancing antioxidant activity and reducing oxidative stress in the diabetic rats. This could be due to the compounds in the MLE, such as flavonoids and phenolic acids, modifying enzyme catalytic activity and compensating for diabetes-induced defects in rats. As such, MLE can be regarded as a potent antioxidant, mitigating the effects of free radicals and metabolic abnormalities associated with diabetes.

Keywords: moringa oleifera leaf extract, alloxan, antioxidant activity, free radicals, biochemical parameters

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Introduction

Diabetes is a metabolic disorder characterized by sustained hyperglycemia stemming from impaired insulin production and its function, or both, posing serious consequences (6). A major predicament faced by diabetics is oxidative stress caused by chronic and continuous hyperglycemia, which compromises the function of the antioxidative

defense system and enhances free radical formation (17).

In diabetes, free radicals are produced disproportionately due to glucose oxidation, nonenzymatic protein glycation, and subsequent oxidative breakdown of glycaemic proteins. Free radicals damage pancreatic β -cells and cause insulin resistance. Oxygen free radicals can react with polyunsaturated fatty acids, causing lipid peroxidation (LPO).

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Diabetes has shown an increasing trend over the years, with substantial and costly repercussions for human and economic capital, particularly in low- and middle-income nations (9).

Due to the high cost of drugs and their side effects on patients, medicinal plants could be usefully combined with diabetes treatments in the framework of an optimized cost-effectiveness strategy (7). *Moringa oleifera*, a native species of the Indian subcontinent, is a fast-growing, drought-resistant tree from the Moringaceae family. It is commonly grown for its versatile use. The moringa is considered a miracle tree as its immature seed pods and green leaves have versatile uses as food supplements and medicines due to their high protein content. In addition, they have numerous health properties, including 10 times more vitamins than carrots, 7 times more vitamin C than oranges, 17 times more calcium than milk, and 15 times more potassium than bananas (1).

Moringa has been linked to improved diabetes management. Its leaves are believed to be an effective agent in lowering blood glucose levels soon after consumption. Moringa extracts (aqueous) have demonstrated substantial healing properties and excellent wound healing and are known to protect against liver damage and aid in reducing liver fibrosis (19).

The moringa tree is an excellent source of antioxidants since it produces more than traditional plant-based sources. Extracts from it can provide a variety of components and have antioxidant action both in vivo and in vitro, owing to its flavonoid and phenolic content. Methanol and ethanol extracts of *M. oleifera* leaves from India showed the greatest antioxidant activity, at 65.1% and 66.8%, respectively, and the leaf powder's antioxidant capabilities can help protect against oxidative stress (6). The study by (12) noted that moringa contains chlorogenic acid, gallic acid, kaempferol, and glycosides, which provide positive antioxidant feedback in chronic circumstances and prevent additional damage. Also, naturally synthesized antioxidants are in

great demand to ensure enough supply for customers.

Liver fibrosis is a dynamic and regulated wound-healing response to chronic hepatocellular damage that represents a major medical problem with significant morbidity and mortality. Moringa has remarkable protective effects. Its antioxidant properties may be due to the presence of phenolic compounds. In this respect, moringa seeds contain important bioactive compounds, including glucosinolates, isothiocyanates, thiocarbamates, and flavonoids. These compounds quench ROS, chelate metal ions, and regenerate membrane-bound antioxidants (12). Therefore, this study aimed to investigate the protective effects of *M. oleifera* leaf extract on some biochemical variables induced by diabetes in male rats with alloxan.

Materials and Methods

Sigma Co. (St. Louis, MO, USA) supplied the alloxan. All additional chemicals and kits were of the highest quality and acquired from standard-certified sources.

The trials used 20 male albino rats weighing 180 ± 150 g and aged 12 weeks. Before the experiment, the rats were kept in the laboratory for two weeks, fed a conventional rodent diet, and provided free access to water. The study protocol was approved by the Scientific Research Ethics Committee, Institute of Graduate Studies and Research, Alexandria University, Egypt (No. 1435-123).

The rats were treated for three weeks after being divided equally into four groups ($n = 5$). Group 1 comprised untreated, healthy rats; Group 2 was treated with MLE, wherein they were given 100 mg/kg BW/day orally, as per the guidelines set by (1); Group 3 received an intraperitoneal injection of 120 mg/kg BW; and Group 4 consisted of diabetic rats that received 100 mg/kg BW/day of orally administered MLE.

To induce diabetes, the rats received 120 mg/kg of intraperitoneal (IP) alloxan injections. The temperature of the alloxan was kept at a low 4 °C, and after being dissolved in 0.9% N/S, a dosage as stated in (10) was injected into rats that had been fasting all night.

After 72 hours, diabetes with a glucose level \geq 250 mg/dl was confirmed.

The leaves of *M. oleifera* were taken from the Elgharbya Governorate in Egypt and dried in a van air circulating oven at 35 to 40 °C until they were fully dry. The powder was obtained by grinding the dry leaves. To derive the aqueous extract for this study, 10 ml of boiling water was added to the oleifera leaves for five minutes after filtering twice into a sterile tube through sterile filter paper of 2- μ m pore size. The stock solution of the aqueous extract was freshly prepared for each set of tests according to (1) and stored at 4 °C for up to 5 days at a concentration of 100 mg/ml.

After the experimental procedures, complete necropsies were performed on the test subjects by anesthetizing them with IP injections using a 22–25-gauge needle and sodium pentobarbital at a concentration of 240 mg/ml. Blood samples from each rat were taken from the inferior vena cava. Samples were transferred to non-heparinized glass tubes and coagulated for thirty minutes at room temperature. Centrifugation at 4000 rpm for ten minutes separated the blood serum, and the serum sample was stored at -20 °C for analysis.

As directed by the manufacturer, Spinreact Kit (Barcelona, Spain), serum glucose levels, HbA1c, triglycerides (TG), total cholesterol (TC), total protein (TP), total bilirubin (TB) concentration, high-density lipoprotein

cholesterol (HDL), and albumin (Alb) were measured. MAD, SOD, and GSH levels were determined using a rate ELISA kit (Sunlong Biotech Co., Ltd, www.sunlongbiotech.com, China), and the assay was performed as per the manufacturer's instructions.

Data are presented as mean values \pm SE. One-way ANOVA was used to determine the significant difference among the treatment groups. The biochemical data were considered to be significantly different at $P < 0.05$. All the statistical analyses were done with the SPSS statistical version 21 software package (SPSS® Inc., USA).

Results and Discussion

Table 1 shows that the treatment of male rats with diabetes (alloxan) alone caused a significant ($P < 0.05$) increase in glucose and HbA1c, and C-peptide significantly ($P < 0.05$) decreased in the serum compared to the control group. On the other hand, the group treated with moringa oleifera leaf extract (MLE) alone showed no change in any of the above parameters. While the presence of MLE in combination with diabetes (alloxan) alleviated or minimized the increase in the above parameters, they did not reach the control value levels.

Table 1. Glucose, HbA1c, and C-peptide levels in rats treated with MLE

Parameters Groups	Glucose mg/dl	HbA1c ng/ml	C-peptide ng/ml
	Mean + SD		
Control	C 96.3+6.59	C 6.052+0.716	A 0.6358+0.0290
Moringa	C 87.75+4.01	C 5.824+0.722	A 0.6195+0.0228
Alloxan	A 276.75+8.2	A 12.68+0.758	C 0.44775+0.01426
Alloxan + Moringa	B 138.75+7.73	B 8.26+0.653	B 0.5687+0.0311
P-value	0.00002	0.00001	0.00005
LSD	9.111334	0.901333	0.031503

- Mean values within a column not sharing common superscript letters (A,B and C) were significantly different, $p < 0.05$.

Table 2 shows that treating the rats with diabetes (alloxan) alone caused a significant

($P < 0.05$) increase in triglycerides, cholesterol, very low-density lipoproteins (VLDL), and

low-density lipoprotein-cholesterol (LDL-c). Meanwhile, high-density lipoproteins (HDL-c) changed insignificantly ($P < 0.05$) in serum compared to the control group. On the other hand, the group treated with MLE alone showed no change in any of the above

parameters. While the presence of MLE in combination with diabetes (alloxan) alleviated or minimized the increase in all of the above parameters except HDL-c, which were insignificantly changed, they did not reach the control value levels.

Table 2. TG, total cholesterol, HDL, VLDL, and LDL levels in MLE-treated rats

Parameters Groups	TG mg/dl	Total cholesterol	HDL mg/dl	VLDL	LDL
	Mean + SD				
Control	C 53.6+2.23	C 67.7+3.12	AB 34.5+3.70	C 10.53+0.432	B 22.7+5.58
Moringa	C 48.4+1.57	C 65.0+4.75	A 38.5+3.27	C 9.89+0.325	B 18.7+3.15
Alloxan	A 75.1+3.72	A 85.8+3.47	AB 32.7+3.25	A 15.02+0.751	A 35.0+4.85
Alloxan + Moringa	B 62.5+3.05	B 71.01+1.58	B 30.2+3.42	B 12.49+0.617	A 33.3+5.27
P-value	0.0004	0.00006	0.0067	0.0002	0.001
LSD	3.470099	4.300864	4.306398	0.694066	6.041742

- Mean values within a column not sharing common superscript letters (A,B and C) were significantly different, $p < 0.05$.

As seen in Table 3, the treatment of rats with diabetes (alloxan) alone caused a significant ($P < 0.05$) increase in creatinine. Meanwhile, vitamin D significantly ($P < 0.05$) decreased in the serum compared to the control group. On the other hand, the rats treated only with MLE showed no change in any of the

above parameters. While the presence of MLE in combination with diabetes (alloxan) alleviated or minimized the increase in creatinine and raised the level of vitamin D, they did not reach the control value levels. Also, there was hardly any change in the urea levels for all the treatment groups.

Table 3. Creatinine, urea, and vitamin D in rats treated with MLE

Parameters Groups	Creatinine mg/dl	Urea mg/dl	Vitamin D ng/ml
	Mean + SD		
Control	C 0.483+0.0489	A 38.4+3.97	A 35.8+2.27
Moringa	C 0.445+0.0476	A 37.3+4.60	A 36.4+2.43
Alloxan	A 0.887+0.0238	A 43.1+4.40	C 17.1+1.58
Alloxan + Moringa	B 0.642+0.0242	A 40.1+2.25	B 27.8+0.98
P-value	0.0003	0.212	0.0005

- Mean values within a column not sharing common superscript letters (A,B and C) were significantly different, $p < 0.05$.

Table 4 shows that the treatment of male rats with diabetes (alloxan) alone caused a significant ($P < 0.05$) decrease in total protein (TP), albumin, and globulin in the serum

compared to the control group. Meanwhile, total bilirubin significantly increased, while direct bilirubin showed no change. On the other hand, the group treated with MLE alone

recorded no change in all of the above parameters. While the presence of MLE and diabetes (alloxan) elevated or maximized the decrease in TP, albumin, and globulin, their

values did not reach those of the control group. Still, direct bilirubin showed significant changes in the presence of MLE in combination with alloxan.

Table 4. Total protein, albumin, globulin, total bilirubin, and direct bilirubin levels in the blood serum of the MLE-treated rats

Parameters Groups	Total Protein g/dl	Albumin g/dl	Globulin Mean + SD	Total Bilirubin mg/dl	Direct Bilirubin mg/dl
	Control	A 2.68±0.145	A 5.26±0.303	A 2.58±0.443	C 1.99±0.273
Moringa	A 2.76±0.145	A 5.44±0.362	A 2.68±0.490	C 1.75±0.208	B 0.41±0.058
Alloxan	C 1.49±0.084	C 2.62±0.154	B 1.13±0.086	A 4.35±0.597	A 0.56±0.035
Alloxan + Moringa	B 1.97±0.092	B 4.49±0.138	A 2.53±0.074	B 2.85±0.182	A 0.54±0.032
P-value	0.0002	0.00001	0.00056	0.0004	0.006
LSD	0.15105	0.323962	0.421886	0.447988	0.063929

- Mean values within a column not sharing common superscript letters (A,B and C) were significantly different, p < 0.05.

As noted in Table 5, the treatment of the rats with diabetes (alloxan) alone produced a significant (P<0.05) increase in MDA, while GSH and SOD significantly (P<0.05) decreased in the serum compared to the control group. Alternatively, the MLE-treated group showed no change in MDA and GSH, while

SOD significantly (P<0.05) decreased. Meanwhile, MLE in combination with diabetes (alloxan) alleviated or minimized the increase in MDA and SOD parameters, though their values did not reach those of the control, while there was a significant increase in GSH (P<0.05).

Table 5. Effect on the MDA, GSH, and SOD in the blood serum of diabetic rats treated with MLE

Parameters Groups	MDA ng/ml	GSH pg/ml	SOD pg/ml
	Mean + SD		
Control	E 53.69±4.18	A 726.1±43.74	A 667.1±40.63
Moringa	E 54.66±4.15	A 723±44.9	B 666.1±41.337
Alloxan	A 90±6.33	D 429±55.4	C 505.2±48.47
Alloxan + Moringa	C 71.92±6.54	C 618.1±44.8	D 594.7±41.29
P-Value	0.0013	0.0009	0.00012
Sign.	Sign.	Sign.	Sign.

- Mean values within a column not sharing common superscript letters (A, B, C, D and E) were significantly different, p < 0.05.

Diabetes is a chronic metabolic disorder characterized by high blood sugar levels, which can lead to various complications if left untreated. It is a prevalent health condition worldwide, with an increasing need for alternative treatments to effectively manage it

(19). Moringa oleifera L. extract is produced from the moringa tree leaves and has shown promise regarding its bioactive components and their impact on biochemical alterations related to diabetes. The part of the plant called M. oleifera has uses ranging from almost every

part for culinary purposes; its seeds are added to water that has already been treated for human consumption. This study was inspired by these features of the plant to assess *M. oleifera* tissues as sources of natural antioxidants. Therefore, the ability of antioxidants to reduce the harmful impact of free radicals in the human body has garnered much interest. In such a context, natural antioxidants are preferred over synthetics (15). Besides, as seen in Table 5, diets rich in vegetables and fruits are related to lower disease risks.

The extract of *Moringa Oleifera L.* contains bioactive compounds, including quercetin, catechin, and kaempferol, which have antioxidant and anti-inflammatory properties that could be responsible for protection against diabetes in the male rats studied. This study also established that MLE can modulate biochemical alterations linked to diabetes by improving insulin sensitivity, reducing oxidative stress, and decreasing inflammation, and involves enhancing glucose uptake by cells, preserving pancreatic beta-cell function, and regulating key signaling pathways involved in glucose metabolism.

The administration of MLE simultaneously with alloxan mitigated the rise in glucose and HbA1c levels. These findings are corroborated by research (19) and (6) on *Moringa Oleifera L.*'s activities. The extract has demonstrated promising benefits in changing the metabolic alterations associated with diabetes in male rats. It has been shown to lower blood sugar levels and enhance insulin sensitivity in diabetic rats, as well as reduce signs of oxidative stress and inflammation. For example, (8,14) found that administering the extract effectively reduced blood glucose levels and enhanced lipid profile markers in diabetic rats.

The current findings also revealed that alloxan causes a rise in serum triglyceride, cholesterol, VLDL, and LDL-c levels. MLE treatment in rats relieved or reduced the rise in triglycerides, cholesterol, very low-density lipoproteins (VLDL), and low-density lipoprotein-cholesterol (LDL-c), as seen in Table 2. The "antilipidemic effect of moringa"

in this study is consistent with previous findings. According to (18), *Moringa Oleifera L.* extract can improve lipid metabolism and reduce triglycerides, improving the overall cholesterol profile and boosting HDL-c levels.

Lipids are essential in the human body, with triglycerides and cholesterol as major components affecting cardiovascular health. Triglycerides are energy stores, whereas cholesterol is required for cell membrane construction and hormone production. HDL-c and LDL-c are essential lipid particles that carry cholesterol through the blood circulation. HDL-c is known as "good" cholesterol for its protective effects against heart disease, while LDL-c, often termed "bad" cholesterol, can contribute to plaque buildup in arteries. A study by (16) showed that MLE was more effective in increasing HDL levels, with a yield of 34%, and lowering LDL levels, with a yield of 24%. On the other hand, the leaf extract was less effective in reducing total cholesterol and triglyceride levels in the blood by 21%. According to (2, 13), *Moringa Oleifera L.* (MLE) protected diabetic rats against hyperglycemia, related disorders, and hyperlipidemia through modulation of general metabolism.

Creatinine, urea, and vitamin D are essential biomarkers that play crucial roles in maintaining homeostasis within the human body. Alterations in the levels of these markers can often indicate underlying health conditions, including diabetes. In individuals with diabetes, there is a notable disturbance in the levels of creatinine, urea, and vitamin D compared to those without the condition. Creatinine and urea levels may be elevated due to impaired kidney function, commonly seen in diabetic nephropathy. Low levels of vitamin D have also been associated with an increased risk of developing diabetes and its complications. This study agreed with the current results, as it presented that alloxan caused an increase in creatinine and minimized the level of vitamin D.

The presence of MLE with the effect of diabetes (alloxan) alleviated or reduced the increase in creatinine and raised the level of vitamin D. Further research is required to

elucidate the mechanism underlying the protective effects of MLE against diabetes and its application in diabetic management strategies. Understanding the alterations in creatinine, urea, and vitamin D levels in relation to diabetes might provide a clearer picture of how to improve outcomes for patients with this metabolic disorder through natural treatments such as MLE (3).

Total protein, albumin, globulin, total bilirubin, and direct bilirubin are important biomarkers in the health and diagnosis of several underlying diseases. The amount of protein in the body demonstrates its overall status, which is critical for most physiological functions. Albumin is the most abundant protein in blood plasma, contributing to oncotic pressure and carrying substances such as hormones and drugs. Globulins represent a heterogeneous group of proteins, with their components including everything from antibodies to enzymes that play significant roles in immunity and metabolism. Bilirubin is derived during the catabolism of heme, and its increase has conventionally been associated with disorders of liver dysfunction and hemolytic diseases. Direct bilirubin measures the conjugated bilirubin level and usually finds applications in diagnosing states such as cholestasis (5).

Glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) levels in biological systems are important to antioxidant mechanisms that protect cellular homeostasis. Antioxidants are two of the most important components of the protective system that guards the cell against oxidative stress arising from an imbalance between the formation and detoxification of active forms of oxygen. The current data showed that the treatment with diabetes alone (alloxan) caused a sharp increase in MDA, while GSH and SOD were significantly reduced in the serum. In contrast, in the case of the extract of the *moringa oleifera* leaves, MDA either had no rise or was minimized, while GSH and SOD increased significantly. These results align with the study by (1). The extract has the potential to improve

antioxidant defenses by enzymes such as SOD and GSH.

Glutathione is a tripeptide molecule comprising cysteine, glutamate, and glycine. It is a powerful antioxidant that neutralizes free radicals and other harmful molecules that might otherwise cause cell damage. GSH also takes part in the body's detoxification processes by getting conjugated to toxins, with their further elimination. GSH deficiency has been implicated in several ailments, such as cardiovascular diseases, neurodegenerative disorders, and cancer (11).

Superoxide dismutase is an antioxidant enzyme that converts superoxide radicals into oxygen and hydrogen peroxide, thus decreasing oxidative stress within the cell. Malondialdehyde represents a lipid peroxidation marker, reflecting oxidative damage to cell membranes. While some studies relate changes in SOD activity and increased MDA levels to aging processes, inflammatory conditions, and metabolic disorders, others associate them with conditions such as diabetes mellitus and cardiovascular disease (20). Pancreatic β cells release C-peptide in an equimolar ratio to insulin. C-peptide, unlike insulin, is not removed by the liver or other organs; hence, it more precisely represents endogenous insulin production. C-peptide serves as a marker of β -cell function and has been primarily used to assess the presence of an insulin-dependent state in diagnosing type 1 diabetes (4).

Insulin, a peptide hormone generated by β -cells in pancreatic islets, is essential for glucose metabolism. During insulin generation and release, the precursor molecule known as proinsulin is converted to proinsulin by signal peptide cleavage. Then, the proinsulin matures into active insulin by the action of cellular endopeptidases, releasing C-peptide and leaving the insulin A- and B-chains linked by two disulfide linkages. C-peptide was discovered in 1967 and was previously thought to be an inert byproduct of insulin production (5).

Conclusion

Moringa oleifera leaf extract (MLE) has the potential to protect diabetic mice from alloxan as it prevents the action of reactive oxygen species (ROS) and has anti-inflammatory and antioxidant activities. Therefore, clinical trials are strongly indicated to determine whether it has a similar effect on antioxidant enzymes so that animal and human trials can be correlated more effectively.

Supplementary Materials

None

Author Contributions

Author 1: methodology, writing, and original draft preparation; authors 2, 3, and 4: writing review and editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study protocol was approved by the Scientific Research Ethics Committee, Institute of Graduate Studies and Research, Alexandria University in Egypt (No. 1435-123).

Informed Consent Statement

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Data Availability Statement

None.

Conflicts of Interest

The authors declare no conflict of interest.

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مستخلص *Moringa Oleifera L*. يعدّل الإجهاد التأكسدي والتغيرات الأيضية المرتبطة بمرض السكري في ذكور الجرذان

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الخلاصة

داء السكري هو اضطراب أيضي مزمن يتميز بفرط سكر الدم المستمر، وينتج عن الإجهاد التأكسدي وتكوين الجذور الحرة، مما يؤدي إلى تلف خلايا β في البنكرياس، وينتهي بمقاومة الأنسولين مما يفاقم الحالة المرضية. تبحث هذه الدراسة في تأثير مستخلص أوراق *Moringa oleifera* على التغيرات البيوكيميائية في ذكور الجرذان المصابة بالسكري. تم إجراء التجربة حيث تم تحفيز مرض السكري في الجرذان باستخدام الألوكسان، ثم تم علاجها بمستخلص (MLE). تمت دراسة المعايير البيوكيميائية الأساسية، بما في ذلك مستويات الجلوكوز، HbA1c، دهون الدم، مؤشرات وظائف الكلى، ومضادات الأكسدة. أظهرت النتائج أن MLE أدى إلى انخفاض ملحوظ في مستويات ارتفاع السكر في الدم ومؤشرات الإجهاد التأكسدي لدى الجرذان المصابة بالسكري. كما أدى إلى تقليل مستويات الجلوكوز، HbA1c، دهون الدم، ووظائف الكلى بشكل كبير، مع زيادة نشاط مضادات الأكسدة وتقليل الضرر التأكسدي. تشير هذه النتائج إلى أن المركبات النشطة بيولوجيًا في مستخلص المورينغا، مثل الفلافونويدات والأحماض الفينولية، قد تكون مضادات أكسدة فعالة للغاية، مما يساعد في معادلة الجذور الحرة ومنع التغيرات الأيضية الناتجة عن مرض السكري. توصلت الدراسة الحالية إلى أن *Moringa oleifera* يمكن أن يكون عاملاً طبيًا طبيعيًا واعدًا للسيطرة على مرض السكري، مما يوفر خيارًا اقتصاديًا وأمنًا مكملًا للعلاجات التقليدية. ومع ذلك، هناك حاجة إلى مزيد من الأبحاث لتوضيح الآليات المتضمنة واستكشاف التطبيقات العملية في المرضى المصابين بالسكري.

كلمات مفتاحية: مستخلص أوراق (MLE)؛ Alloxan، مضاد للأكسدة، الجذور الحرة، القياسات الكيميائية الحيوية

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