International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 19-10-2022, Accepted: 04-11-2022, Published: 22-11-2022
Volume 7, Issue 11, 2022, Page No. 72-75

Study of the effect of aqueous extract of *Moringa oleifera* leaves and nano selenium on glucose level and liver enzymes in male rats experimentally infected with diabetes

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Abstract
The present study aimed to evaluate the effectiveness of Moringa oleifera extract and nano selenium in reducing the levels of hepatic enzymes (AST, ALT) in diabetic rats. This study was conducted in the animal house / College of Science / University of Kufa and the study extended from 1/12/2021 to 1/6/2022. The study included the use of 250 mg/kg of MO and 0.4 mg/kg of SeNPs separately and a mixture of The two substances together as a combined treatment. The results showed a decrease in the level of glucose and liver enzymes in the groups treated with MO and SeNPs. The results also showed the highest therapeutic efficiency when using the two substances together as a joint treatment. The results of the histological study showed therapeutic effects on the liver after treatment with MO and SeNPs. We conclude from this study that Moringa oleifera and SeNPs are effective in treating diabetes.

Keywords: moringa oleifera, nano selenium, diabetes mellitus

Introduction
Diabetes is considered one of the most important and most serious public health problems due to the large number of injuries and the high death rate, as it occurs as a result of a disorder in metabolic processes due to many pathogens, which results in a high level of sugar in the blood of people with diabetes due to the inability of their bodies to produce enough insulin. or respond to it (Jian *et al.*, 2020) [9]. Moringaoleifera is one of the most important medicinal plants belonging to the family of Moringaceae and characterized by its high medicinal and nutritional value. It is native to India and is cultivated in tropical and subtropical regions, and later it began to be cultivated all over the world (Premi and Sharma, 2013; Radovich, 2010) [17]. It was known as the miracle tree because of its many benefits and therapeutic uses.
Nanotechnology is one of the modern innovations that can be defined as the process of preparing, designing and characterizing nanomaterials with changes in their chemical and physical properties, including shape and size as well as their crystalline nature (Han *et al*., 2016; Raza *et al*., 2016; Cacciotti *et al*., 2018) [6, 20, 2]. In recent years, the process of manufacturing nanoscale selenium has been increasingly given attention due to its advantages, including its low cost, many sources of raw materials, and its safe toxicity. It is also considered an environmentally friendly material (Husen and Siddiqi, 2014) [7].

Materials and Working Methods
Experimental Animals
25 animals were purchased from male rats of the type Rattusrattus at the age of 12 weeks and weighing 180-250 g. The animals were transferred from one of the animal houses in the city of Hilla to be worked on in the animal house of the College of Science / University of Kufa, they were raised under the appropriate environmental conditions in terms of degree Heat, ventilation, provision of drinking water and an appropriate diet. They were placed in cages at a rate of 5 mice in each cage and in 5 cages.

Preparation of selenium nanoparticles, SeNPs
At the beginning of the process, lemon peel extract weighing 3 g of lemon peel powder is prepared with 100 ml of distilled water. It was placed in a glass beaker with a capacity of 200 ml and transferred to the water bath at a temperature of 70 ° C for two hours, then left to cool at a laboratory temperature of 25 ° C. Then the extract is filtered by white medical gauze into a glass beaker to get rid of the impurities to be placed in a centrifuge 4500 rpm for 15 minutes. The extract was separated from the precipitate and 0.004 sodium selenite was added to the extract. Then it was placed on a magnetic stirrer at a temperature of 70 °C at 600 cycles per minute for a period of 5 hours until the color of the extract changed from yellow to ruby red (Sowmiya *et al.*, 2014).

Characterization of selenium nanoparticles
Selenium nanoparticles were characterized by UV-vis spectrophotometer, infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis.

Estimation of glucose level in blood serum
For the purpose of measuring the level of blood sugar, the following method was adopted using a test kit from the company (Spinreact, Spain) (Tietz, 1995) [22].

$$\text{Glucose concentration (mg/dl)} = \frac{\text{(A) Sample}}{\text{(A) Standard}} \times \text{Standard Conc. (100 mg/dl)}$$

Determination of the level of alanine aminotransferase and aspartate aminotransferase (AST, ALT) in blood serum:

The enzymatic method was relied on to detect the concentration of AST, ALT in samples using a special analysis kit for this purpose (Kit) from Biomerieux Company (Tieza, 1995).

L-Alanine + 2-Oxaglutarate ⟶ Pyruvate + L-glutamate

L-Aspartate + Oxaglutarate ⟶ Oxalacetate + L-glutamate

Development of Diabetes

The animals were starved for 24 hours and then injected with a solution of alloxan at a concentration of 120 mg/kg of body weight, in a single dose under the skin (Rashid *et al*., 2013) [19] and then given a 5% glucose solution to avoid their death. After 3 days, the animals showed a level of more than 200 mg/kg of body weight. dl of glucose and she was considered diabetic.

Animal Treatment

The experimental animals were divided into 5 groups for each group of 5 animals: the first group was the control and the second group was treated with alloxan, the third group was treated with alloxan and Moringa oleifera at a concentration of 250 mg/kg per 1 ml (Akinrinde *et al*., 2020) [1], the fourth group was treated with nanoselenium at a dose of 0.4 mg/ How many ml is 1 ml (Loeschner *et al*., 2014) [11], the fifth group treated with Moringa oleifera and nano selenium.

Results

The results showed a significant increase (P<0.05) in body weight in the control group, and a significant decrease in the group of animals treated with alloxan, as shown in Table (1). Also, a significant increase (P<0.05) appeared in the groups of other animals treated with Moringa oleifera and nano selenium. The results in the table showed a significant increase (P<0.05) in the level of glucose in the alloxan-treated rats in Hasan. There was a significant decrease (P<0.05) in The two groups of rats treated with MO and SeNPs, respectively, and there was a significant decrease (P<0.05) in the group of rats treated with MO and SeNPs together as a combined treatment. There was a significant increase (P<0.05) in the activity of liver enzymes: ALT, AST in the control group. Positive treatment with alloxan compared with the negative control group, and there was a significant decrease (P<0.05) in the same enzymes in the two groups of infected rats treated with aqueous extract of Moringa oleifera and nano selenium each separately, as well as the group of infected rats treated with aqueous extract of Moringa oleifera and nano selenium as a combined treatment. Compared with the infected positive control group. As in Table (2).

Table 1: Effect of Alloxan, Moringa Oleifera Extract and Nano-Selenium on body weight

The group	Weight before treatment (g)	Weight after treatment (g)	Weight difference (g)
Alloxan	209.75 ± 10.39a	134 ± 18.76 a	-75.7 ± 16.5 a
Alloxan+Mo	222.5 ± 7.59ab	203 ± 11.37 b	-19.5 ± 4.93b
Alloxan+ SePNs	223.25 ± 8.05b	225 ± 6.63c	1.75 ± 6.13 c
Alloxan+Mo+ SePNs	229.75 ± 11.7b	253 ± 5.29d	23.25 ± 7.36 d
Control	248.75 ± 5.61c	295.2 ± 10.04e	46.5 ± 6.60 e
LSD	13.48	17.24	14.01

Table 2: Effect of alloxan, Moringa oleifera extract and nano selenium on glucose level in healthy and and liver enzymes (AST, ALT) in experimentally diabetic white rats.

The group	Glucose Level (mg/dl)	AST (U/L)	ALT (U/L)
Alloxan	413.25 ±31.4 a	166.75 ±9.58 a	117.25± 7.29 a
Mo+Alloxan	122.5 ± 2.12 b	53.50 ± 4.17 b	50.0 ± 3.13 b
SePNs+ Alloxan	116.0± 2.94 b	49.75 ± 3.27 b	43.75±4.49 bc
Mo+ SePNs + Alloxan	95.75 ± 2.72 b	40.75± 4.49 b	41.75± 4.87 bc
Control	99.5 ± 0.65 b 99.5 ± 0.65 b	39.75 ± 4.51b	33.00±3.43 c
LSD	13.988	10.074	8.293

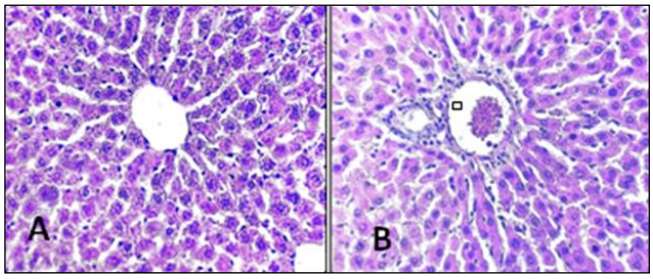

Fig 1

Figure (A) section of the liver tissue in the control group, which shows a central vein surrounded by cells. (40X). (B) A histological section of the liver of rats treated with alloxan, lysis and necrosis of hepatocytes, hemorrhage into the central vein, and infiltration of inflammatory cells. (40X).

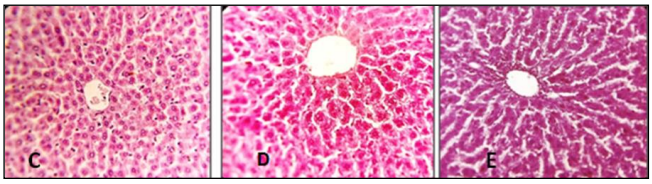

Fig 2

(C) Histological section of the liver in the group treated with Moringa, which shows improvement in the tissue with shrinkage in the central vein Figure (D) Histological section of the liver in the group treated with nano-selenium showing normal cells around the normal central vein Figure (E) Histological section of the liver in the group of rats treated with Moringa and Selenium, which appears normal and identical to the control group.

Discussion

In the current study, alloxan showed a satisfactory effect by raising the level of sugar and liver enzymes and destroying the tissue of cells, which explains that alloxan causes liver disease through disturbance in metabolic processes and enlargement of hepatocytes, which leads to increased production of hepatic enzymes and destruction of renal tissue (Chaudhary *et al*., 2018) [3].

It was found that the leaves are the most important and beneficial part of the tree because of their high content of active substances compared to the rest of the plant, such as proteins, carbohydrates, carotenoids, saponins and tannins (Oladeji *et al*,. 2017) [15]. And that these substances are what gives the plant its medicinal and nutritional importance (Pandey *et al*., 2019) [16]. Researches and studies have also proven the importance of the aqueous extracts of the Moringa tree, which possesses a strong bio-antioxidant activity and works to remove free radicals, including the aqueous extract of the seeds, which is characterized by its high content of flavonoids compared to extracts of methanol and acetone. (Jahan *et al*., 2018) [8]. The current study benefited the ability of Moringa leaves and their effective role in reducing levels, improving liver function and lowering blood sugar, which is attributed to the phytochemical content in Moringa leaves and their antioxidant potential (Mabrouki *et al*., 2020) [12].

Increased levels of ALT and AST have been associated with increased gluconeogenesis during diabetes (Nabi *et al*., 2013) [14]. However, this study showed that treatment with SeNPs restored ALT and AST activity toward normal levels. This result can be explained by the radical scavenging property of Se and its importance in protecting tissue integrity and function (Messarah *et al*., 2012) [13]. In a study conducted by (Guan *et al*., 2018) [5] it was confirmed that Se-NPs have a substantial effect on blood sugar along with their antioxidant and anti-inflammatory activities, so T1DM and T2DM can be treated with Se-NPs by alleviating oxidative stress and insulin sensitization. This effect may be due to the ability of selenium to regenerate beta-cell activity, stimulate insulin secretion, and lower the level of glucose in the blood (Eskander *et al*., 2000) [4]. This is consistent with the results of (Liu *et al*., 2018) [10] who reported that the use of Se It has a protective role against diabetes mellitus by improving blood sugar, restoring normal body weight, relieving oxidative stress, and treating dyslipidemia in diabetic rats.

Conclusions

1. Moringa oleifera extract had the effect of alleviating the complications of diabetes, and it did not have any harmful effects in experimental animals.
2. The strong efficacy of selenium nanoparticles as antioxidants has been demonstrated.
3. Selenium nanoparticles combined with Moringa oleifera extract has a more prominent effect on the values of biochemical indicators and tissue damage associated with diabetes than nanoparticles of selenium or Moringa oleifera as a single treatment.

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