

Evaluation of the therapeutic effect of Nano-Selenium-encapsulated insulin on some liver enzymes in induced diabetic rats

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ABSTRACT

Nanotechnology, specifically nanocarriers, has demonstrated remarkable results in the treatment of diabetes in animal models, as this approach is distinguished by many unique physical, chemical, and biological properties of nanomaterials that enhance drug delivery and therapeutic effectiveness on insulin delivery for diabetes treatment. The study aimed to assess the therapeutic impact of selenium-insulin nano-envelopes. This envelope was examined using UV-vis measurements in the wavelength spectrum of (208.50-407.50) nm, as well as XRD, the specimens were next examined using (FESEM) as spherical particles with nano-diameters ranging from (110-40) nm. The rats were placed into three sets: a control, a diabetic, and a diabetic treated with the selenium-insulin nanocapsule. The infected control group showed AST values of 155.1 ± 678.5 , ALT values of 67.20 ± 179.3 , and ALP values of 202.7 ± 444.0 . The findings revealed that the untreated diabetic group had significantly higher liver enzyme levels, indicating liver damage. The nano-encapsulated group experienced a significant drop in enzyme levels, including AST (6.18 ± 161.0), ALT (6.14 ± 60.50), and ALP (72.71 ± 627.8), indicating liver protection. These findings show that selenium-insulin nanoencapsulation could play an essential role in protecting the liver from diabetes-induced damage, and allowing for greater insulin absorption and protection from degradation. Histological examination of the liver in the healthy control group showed intact hepatic cords and central vein, while congestion of the central veins and venous sinuses was observed in the alloxan-induced diabetic group. In the diabetic group treated with the selenium nanocapsule, significant improvements were observed in hepatocyte levels and the hepatic central vein.

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1. INTRODUCTION

Diabetes is the most prevalent endocrine ailment, impacting about 100 million individuals globally (6% of the total population). It is caused by the pancreas' inability or failure to produce insulin, resulting in elevated or decreased blood glucose concentrations. It has been shown to harm numerous body systems, particularly the blood vessels, eyes, kidneys, heart, and nerves [1]. Diabetes is linked to a number of malignancies, including liver, pancreatic, and endometrial [2]. Diabetes complications such as heart attacks, strokes, renal failure, amputation, and blindness result from a failure to recognize and treat the disease early [3]. Diabetes is divided into two types: insulin-dependent diabetes (IDDM) and non-insulin-dependent diabetes (NIDDM), insulin-dependent diabetes is an autoimmune illness defined by a local inflammatory response in and around the pancreatic islets, followed by selective death of insulin-secreting cells, whereas insulin-dependent diabetes is distinguished by peripheral insulin resistance and decreased insulin secretion [4]. In 2021, data indicated that the prevalence of diabetes in Iraq reached 10.7%, reflecting a significant increase in cases over the past few years [5]. Medications are generally used to save lives and treat ailments. Secondary objectives include preventing long-term diabetic issues and increasing lifespan through the elimination of numerous risk factors.

Type 1 diabetes is treated primarily with insulin replacement medication, but type 2 diabetes is treated and managed mostly by dietary and lifestyle changes [6]. Nanotechnology is a novel and sophisticated approach for providing nanotherapeutics in medicine delivery to treat diabetes. Nanotechnology-based nanocarriers include revolutionized pharmaceutical research and development by allowing for exact manipulation of form, particle size, surface characteristics, and the liberate pharmaceutically effective substances at specified targeted sites [7,8]. Nanotechnology in diabetes research has permitted the development of new technologies for detecting glucose levels and delivering insulin, allowing diabetic patients to enhance their quality of life significantly. Our focus is on current advances in diabetes research and their interactions with nanotechnology [9]. The use of nanoparticles can address issues such as brief half-life, limited enteric permeability, and absorption linked with conventional diabetic medicines [10]. As a result, nanoparticles have evolved as additional appropriate, secure, and non-intrusive way of insulin administration to address these limits in diabetes care [11]. Selenium nanoparticles, in addition to being antioxidants and anti-inflammatory, have a hypoglycemic effect. Thus, selenium nanoparticles can treat insulin-dependent diabetes and non- Insulin-dependent diabetes by reducing imbalance of free radicals and antioxidants in the body and enhancing insulin sensitivity [12].

Previous investigations have shown that selenium nanoparticles had a hypoglycemic impact in streptozotocin induced type 1 diabetes, resulting in a considerable reduction in glucose levels, higher insulin levels, normalization of liver and kidney function, and an improved lipid profile [13].

2- METHOD

2.1 The biosynthesis of selenium-insulin nanocapsules

Insulin was used to create composite selenium nanoparticles, as previously described [14]. Insulin was employed to bioencapsulate selenium nanoparticles in an eco-friendly biochemical process. A combination was created by adding 10 ml of insulin to 90 mL of a 2 millimolar Na₂SeO₃ solution. For the control specimen, 10 mL of pure water was mixed with 90 mL of 2 millimolar Na₂SiO₃ solution. To ensure a uniform mixing, the two vials were brood in a rotating shaker for 3h in the shade . The resultant selenium nanoparticles were separated and purified by centrifugation. The dried selenium nanoparticles were kept at room temperature for subsequent examination. The recombinant human insulin used in this study was obtained from Eli Lilly and Company (USA). The selenium compounds were obtained from Merck.

2.2 Characterization of the Selenium-insulin Nanocapsule.

UV-Vis 1800 spectroscopy was used to characterize the selenium-insulin nanocapsules. dual-beam ultraviolet-visible (UV-Vis) spectrophotometer (PD-303 UV) was employed to characterize the surface plasmon resonance (SPR) properties of selenium–insulin nanoparticles at room temperature. The nanocrystalline structure of the particles was analyzed using X-ray diffraction (XRD) with a Shimadzu XRD-6000 instrument, utilizing Cu-K α radiation ($\lambda = 0.15418$ nm) operated at 40 kV and 30 mA. The average crystallite size was calculated based on the Debye–Scherrer equation: $D = k\lambda / (\beta \cos \theta)$, where D denotes the crystallite diameter, k is the shape factor (0.94), λ is the X-ray wavelength, β represents the full width at half maximum (FWHM) of the diffraction peak, and θ is the Bragg angle. The surface morphology and structural characteristics of the nanospheres were further examined using a field emission scanning electron microscope (FESEM, JEOL JSM-6460LV).

2.3 Experimental Animals

The study used male Wistar rats, conducted at the Biotechnology Research Center at Al-Nahrain University. The study lasted 30 days and was divided into three groups, each containing five rats. The first group was healthy and non-diabetic; the second group was alloxan-induced diabetic at a dose of 150 mg/kg body weight [15]; and the third group was alloxan-induced diabetic and received treatment with selenium-insulin nanocapsules at a dose of 0.3 mg.

2.4 Enzyme tests

This includes

A- Determine the activity of serum's two aminotransferase enzymes, Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST). A method for determining the amount of pyruvate and oxaloacetate released during their interaction with dinitrophenylhydrazine was utilized [16].

B- Assessing serum alkaline phosphatase (ALP) activity. This method is based on determining the amount of phenol produced during its interaction with 4-aminoantipyrine [17].

2.5 Preparation of Tissue Sections

Tissue sections were prepared using the procedure suggested by [18,19]. The fixative was removed by washing the rat liver samples with 70% ethyl alcohol until the yellow tint was gone. Then the stages of leaching, clarifying, filtration, embedding, sectioning, staining, and mounting were completed.

2.6 Statistical Analysis

The obtained data were statistically analyzed using an unpaired t-test via GraphPad Prism version 6 software. Results are expressed as the arithmetic mean $\pm$ standard error of the mean (SEM). Statistical significance was considered at a p-value less than 0.05[20].

3. Results and Discussion.

3.1 UV/Vis Spectroscopy

Figure (3-1) depicts the absorption spectra obtained from UV-vis investigation of the insulin-selenium nanoenvelope. Ten absorption peaks emerged between 208.50 and 407.50 nm, indicating the complicated chemical makeup of the resultant nanoparticles. These findings were congruent with those reported by [21,22]. The peaks at 208.50-243.50 nm demonstrate that insulin absorbs UV radiation due to the presence of amide and peptide bonds in its structure. The peaks at wavelengths (256.50 - 313.50) nm indicate the interaction of nano-selenium and insulin, whereas the farther peak (407.50) nm indicates the presence of a plasmonic effect caused by nano-selenium particles, which supports the interaction of selenium with insulin in the composite nanostructure. This wavelength variety suggests structural changes caused by the interaction of molecules, culminating in the production of a novel nanocomposite with unique optical properties. This is a strong signal of the envelope's nano-formation success and component uniformity. This investigation is consistent with the findings of [23].

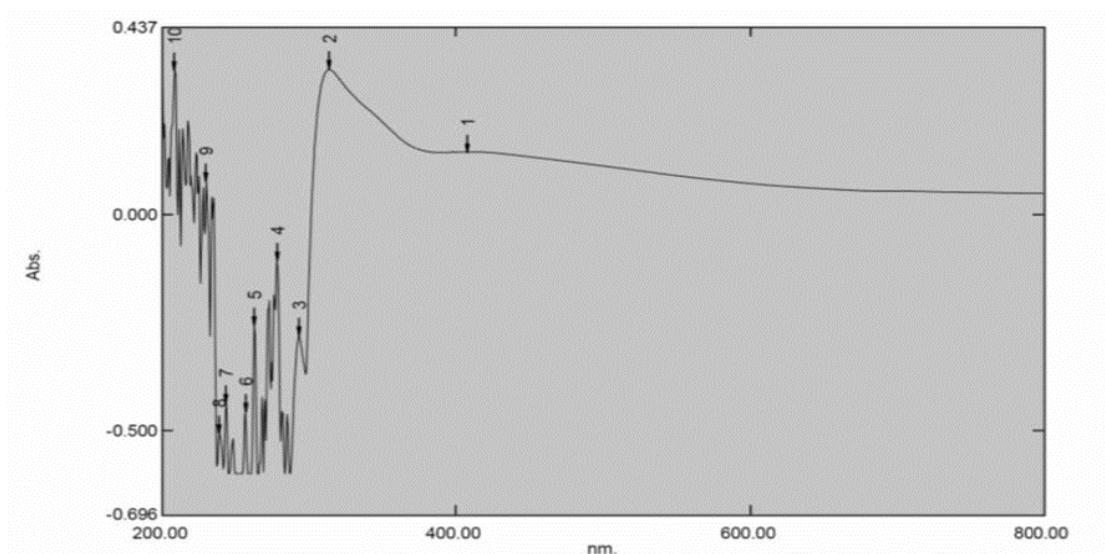


Figure (1) UV-vis spectroscopy of the selenium-insulin carrier

3.2 X-ray diffraction(XRD)

The X-ray diffraction patterns of the selenium-insulin nano-encapsulated material were examined by detecting the peak sites. We found the peak locations at the levels [(100), (101), (110), (102), (111), (200), (201)]. As shown in Figure (2), the polycrystalline membrane and preferred peaks for the formation of crystalline grains were compared to Joint Committee on Powder Diffraction Standards (JCPDS) (0362-06). According to Debye-Scherrer equation calculations, the crystallite size of the nanoparticles of the insulin-bound selenium nanocapsule was estimated to be approximately 44.4 nm at the sharpest peak (at $2\theta \approx 48.8^\circ$), and the findings showed a satisfactory match. The peaks and results also corresponded to several studies, including what the researchers discovered [24]. The sharp, high peaks in the X-ray diffraction (XRD) spectrum indicate a high degree of crystallinity, demonstrating a relatively regular crystalline structure of the nanocomposite. However, the presence of peaks with varying full width and half height (FWHM) indicates some dispersion in crystal size, which is common in synthesized nanoparticles [25]. These crystalline properties play an important role in the compound's stability and biological efficacy, as the degree of crystallinity influences drug release, bioactivity, and tolerance in biological media. Therefore, the calculated crystal size and high degree of crystallinity support the potential use of this formulation as an effective insulin carrier [26].

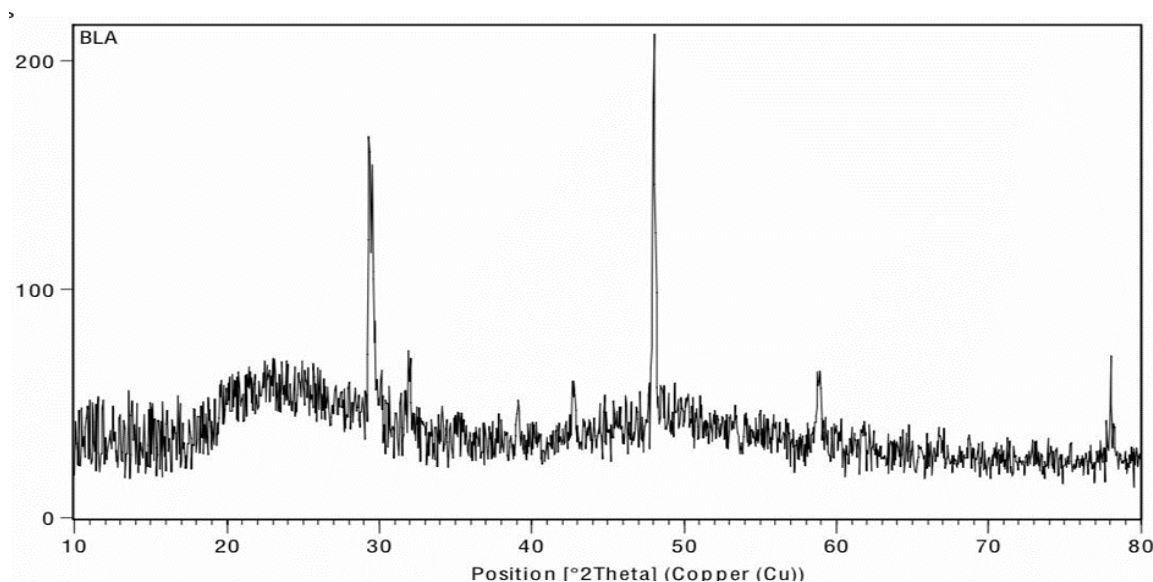


Figure (2) shows an X-ray examination of the selenium-insulin transporter.

3.3 Field-emission scanning electron Microscope (FESEM)

Figure (3) depicts the selenium-insulin nano-encapsulated particles as spherical particles with nano-diameters ranging from 40 to 110 nm, scattered in a semi-regular way with occasional clumps in various places. The total surface was reasonably smooth, indicating that the selenium-insulin encapsulation process was successful at the nanoscale, the nanoparticles showed an average size of approximately 75 nm with a standard deviation of ± 20 nm, as determined by FESEM analysis, these findings were congruent with those of [27]. The molecular size of nanospheres is a crucial factor in determining their bioavailability and pharmacological efficacy, especially in therapeutic applications. Sizes below 100 nanometers are ideal for drug delivery, allowing nanospheres to easily pass through microcapillaries and enter target cells via phagocytosis, without requiring rapid clearance by the liver or kidneys [28]. Studies indicate that coatings in this range also have improved properties in terms of slow drug release, enabling precise, time-controlled insulin delivery that mimics natural secretion. This size is also suitable for avoiding acute immune responses, which is critical in the treatment of chronic diseases such as diabetes [29].

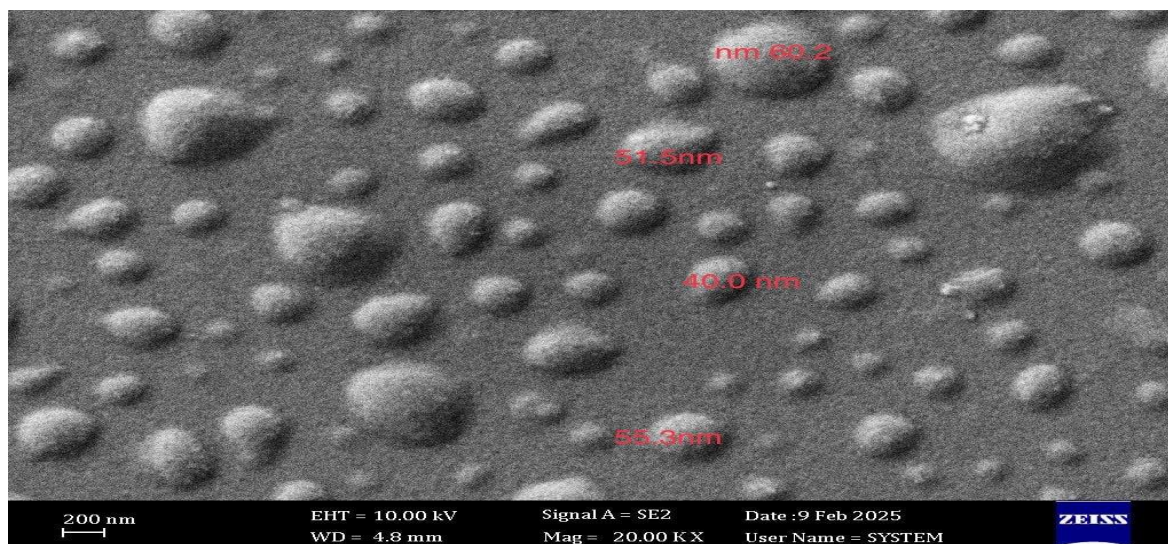


Figure (3) shows a Field-emission scanning electron Microscope (FESEM) examination of the selenium-insulin carrier.

3.4 Estimating Liver Enzyme Levels in Serum of Experimental Animals

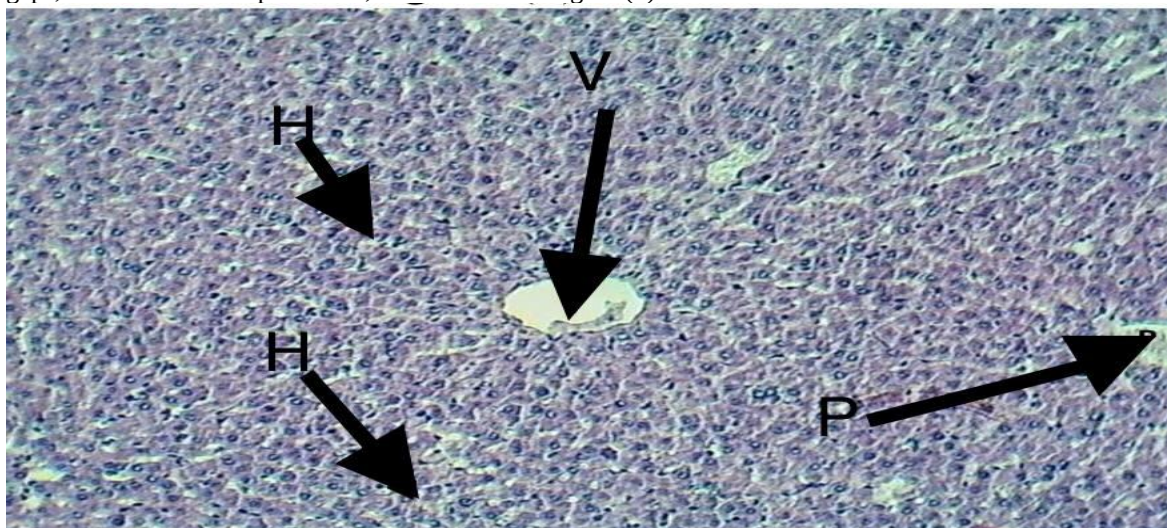
Table (1) shows that the healthy, uninfected control group had significantly lower levels of AST (6.84 ± 105.8), ALT (8.62 ± 69.50), and ALP (26.94 ± 343.0) IU/L compared to the alloxan-induced infected control group, where AST values were (155.1 ± 678.5) and ALT values were (67.20 ± 179.3). The selenium-insulin nano-encapsulated group showed significantly lower levels of AST (6.18 ± 161.0) and ALT (6.14 ± 60.50) enzymes compared to the untreated control group, with ALP enzyme values (202.7 ± 444.0). ALP enzyme levels (72.71 ± 627.8) showed a substantial rise. The current investigation found that alloxan-induced diabetes in rats can produce a rise in serum glucose levels, which is similar with a study conducted by [30]. This increase in diabetic rats is due to damage to pancreatic beta cells caused by alloxan induction [31]. This study supported the findings of [32] in his work on the hepatoprotective impact of selenium in rats induced with diabetes using alloxan, which found that the use of selenium supplements resulted in a significant drop in the levels of liver enzymes such as ALT, ALP, and AST. The findings also revealed an improvement in the liver's tissue structure as well as crucial markers. [33] also demonstrated that treating diabetic rats with nano-selenium can restore normal liver enzyme levels, indicating a liver protective function. Nano selenium serves as a catalyst for the revitalization of selenoproteins such as glutathione peroxidase (GPXs) [34]. Glutathione peroxidase is necessary for cellular protection against oxidative damage to cytoplasmic components [35]. Elevated ALP values and lower AST and ALT levels imply less liver cell damage. ALP levels remain increased, suggesting that the liver is still in the process of repair and regeneration [36]. Additionally, raised ALP levels indicate that this enzyme is passing through the bile duct at a rapid rate. This rise is only transitory and is caused by the bile duct's response to treatment [37].

Table (1) Average levels of some liver enzymes in rats with diabetes

Experimental groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	$105.8^c \pm 6.84$	$69.50^a \pm 8.62$	$343.0^a \pm 26.94$
Diabetic control	$678.5^a \pm 155.1$	$179.3^a \pm 67.20$	$444.0^a \pm 202.7$
Diabetes+Selenium	$161.0^{bc} \pm 6.18$	$60.50^a \pm 6.14$	$627.8^a \pm 72.71$

3.5 Histological Study of the Liver

The results of the transverse histological section of the liver of male rats from the healthy, uninfected control group are presented. It is noticed that it is composed of several lobules, each of which has a central vein surrounded by polygonal cells (hepatocytes) arranged in strips. Between these strips are sinusoid blood gaps, which contain Kupffer cells, as illustrated in Figure (4).



liver tissue from healthy, uninfected male rats showing a normal central vein (V), normal hepatic (H) and portal triad (P) Figure (4) A cross-section of cords. H&E 400x.

Furthermore, the histological analysis revealed in the transverse tissue slice of the liver of male rats from the control group infected and caused with diabetes by alloxan. In multiple areas of the liver lobules, there was blood congestion in the central veins and venous sinusoids, as well as sinusoidal irregularity, which was caused by local damage represented by necrosis in some hepatocytes, thickening of their nuclei (pyknosis), membrane dissolution, the presence of mononuclear inflammatory cells, and mental degeneration in hepatocytes with cytoplasmic rupture, when compared to the unaffected control group. As in Figure (5).

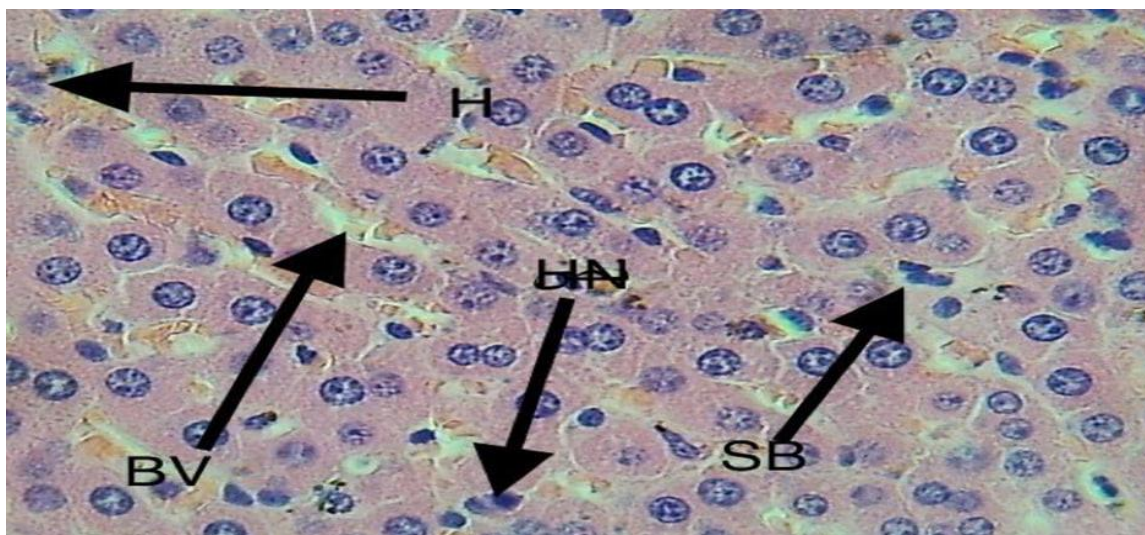


Figure (5) A cross-section of liver tissue from male rats induced with diabetes, Hepatocellular damage (H), steatosis (SB), nucleolar necrosis (NH), and vascular congestion (BV) are seen. H&E 400x.

The current investigation found that inducing diabetes with alloxan causes alterations in the liver of male rats, which is compatible with the work conducted by [38]. Liver cell necrosis occurs as a result of insufficient blood flow to the liver caused by arterial blockage and thrombosis in the hepatic artery, resulting in a shortage of oxygen. This deficiency causes the release of lytic enzymes and secretory chemicals, resulting in liver cell destruction [39]. Furthermore, the existence of blood congestion in some locations is owing to poor blood drainage caused by venous obstruction, which prevents blood from moving through the liver parenchymal cells [40]. The liver tissue regions were examined after a month of treatment with the selenium-insulin nanoencapsulated medication. The results indicated a considerable improvement in hepatic cells. The central hepatic vein and hepatic cords were also shown to be significantly better as compared to the untreated control set. This investigation is congruent with the findings of [41], as illustrated in Figure (6).

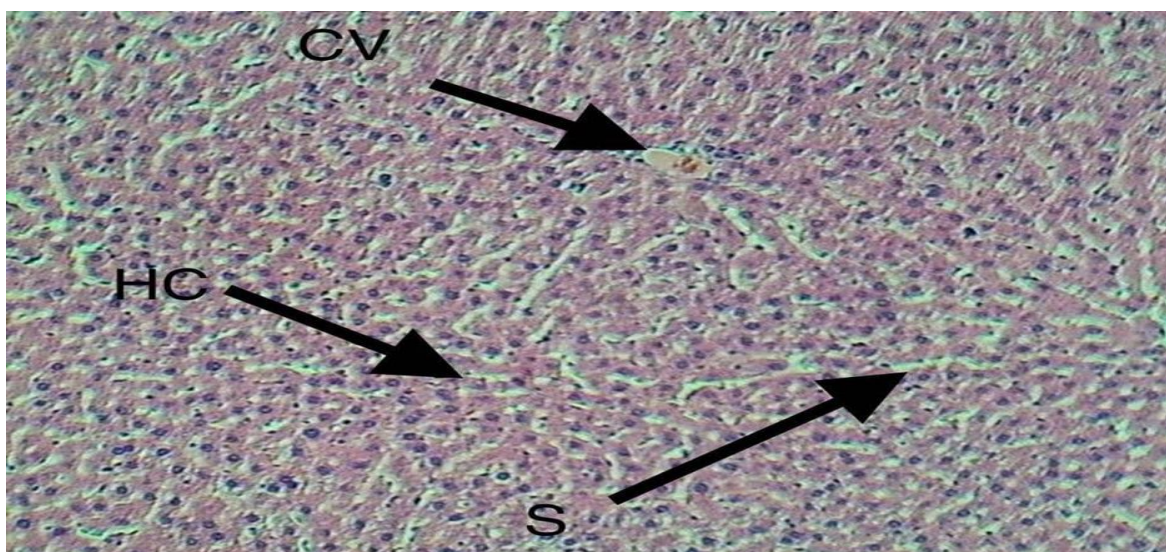


Figure (6). A cross-section of liver tissue from male rats induced with diabetes and treated with nano-selenium with insulin, showing the normal central vein(CV) and normal hepatic cords(HC) and sinusoids (S). H&E. 100x.

4- Conclusion

The findings showed that the use of transformational nanomaterials (NPs), such as nanocapsules containing insulin and selenium, is a valuable successful medication against the treatment of liver damage caused by alloxan-induced diabetes. The treatment led to a significant reduction of liver enzymes (ALT, AST and ALP) with grafted rats, enjoying a more intact livers' histology, which suggests a hepatoprotective effect of these particles to hepatic cells. According to the above, we know that the insulin-nanoselenium is one of the good nano-materials for medical purpose, treating diabetes for medical purposes, and its multiple therapeutic mechanisms give its biological security, than other nano-materials that may have no such balance between efficiency and poison.

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