# Efficacy of selenium nanoparticles 10% suspension on the kidney of diabetic rats

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**Abstract**

**Background:** Diabetes mellitus (DM) is a prevalent non-communicable disease with profound impacts on health and productivity globally. With the majority of diabetic patients facing debilitating complications, urgent measures are warranted, particularly in low- and middle-income countries where the disease burden is high. In recent years, nanotechnology has emerged as a promising avenue in medicine, with selenium nanoparticles garnering attention for their potential therapeutic applications. Selenium, an essential micronutrient, exhibits significant antioxidant properties and plays a crucial role in various physiological processes. Notably, selenium deficiency exacerbates oxidative stress, a hallmark of diabetes, further compromising pancreatic function and exacerbating diabetic complications.

**Aim:** This study investigates the therapeutic potential of selenium nanoparticles in alleviating diabetic complications, particularly nephropathy, in experimental rat models of type 1 diabetes.

**Methods:** The research conducted at the A. Natishvili Institute of Morphology, Tbilisi State University, utilized selenium nanoparticles synthesized through advanced nanomilling techniques. Experimental rats were induced with type 1 diabetes and subsequently treated with selenium nanoparticles, insulin, or a combination of both. Biochemical analyses revealed significant improvements in renal function parameters, including blood urea nitrogen, creatinine, and albumin levels, following treatment with selenium nanoparticles, both alone and in combination with insulin

**Results:** Morphological examinations corroborated these findings, demonstrating reduced inflammatory infiltration and preservation of renal architecture in treated groups compared to untreated diabetic rats. Notably, combined therapy with selenium nanoparticles and insulin exhibited superior efficacy in mitigating renal edema and preserving renal function compared to monotherapy with either agent.

**Conclusions:** These results underscore the potential of selenium nanoparticles as a therapeutic adjunct in the management of diabetic complications, particularly nephropathy. Further research and clinical trials are warranted to elucidate the mechanisms underlying the protective effects of selenium nanoparticles and optimize their clinical utility in diabetic care. **(TCM-GMJ June 2024; 9 (1):P28-P35)**

**Keywords:** Diabetes Mellitus, Selenium Nanoparticles, Kidney

## Introduction

Diabetes mellitus (DM) is considered as one of the most common non-communicable diseases. According to the suggestion of the World Health Organization, diabetes mellitus ranks fifth to sixth among the causes of death in developed countries and ninth to tenth in developing countries [1] DM is certain to be one of the most important health problems in the 21st century. The majority of diabetic patients are living with serious complications that leads to decreased work ability and productivity. Premature death due to diabetes is one of the causes of loss of stable family income. In low- and middle- income countries, where almost 75% of people affected with diabetes live, introduction of urgent and decisive measures against this disease is required [2][3].

In recent years, nanotechnologies occupy an important place. The nanomaterial application paved its way to biology and medicine and is becoming increasingly popular. As for the diabetes mellitus selenium nanoparticles are noteworthy to emphasize in this regard. Selenium is an essential microelement in human body. Organic forms of selenium are present as selenomethionine and selenocysteine and inorganic forms such as selenite and selenate, respectively. Selenium was found to be present in selenium containing proteins, among which glutathione peroxidase (GPX), thyroid reductase (TR) and selenophosphate synthetase (SPS) are of great importance [4]–[7]. The recent studies highlighted a positive effect of selenium on various organ systems. Selenium nanoparticles have strong

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antioxidant, antimicrobial, anticarcinogenic properties, and are characterized with good tissue bioavailability and less toxicity, they can be administered orally [8]–[19]

Selenium deficiency decreases the number of antioxidants glutathione peroxidase (GPx) and consequently leads to an increase in oxidative stress [20]–[25]. Hyperglycemia is also associated with enhanced oxidative stress - due to an increase in superoxide radicals (O2-) and other reactive oxygen species (ROS). In addition, the activity of such antioxidants as: superoxide dismutase (SOD), glutathione peroxidase (GPC) and catalase are decreased, in their turn, reducing antioxidative protection. Selenium (Se), and namely, selenocysteine, is an important component of antioxidant enzymes, such as glutathione peroxidase (GPx). Glutathione (GSH) is one of the most potent antioxidants. A biological function of GPx is to protect the body against oxidative stress. After interaction with free radicals glutathione (GSH) reverts to its oxidized form (GSSG) [4]–[7], [26]. Pancreas is extremely vulnerable to selenium deficiency. Selenium deficiency proved to affect the structure and function of pancreas [27]. It should be emphasized that pancreatic beta cells are one of the least provided cells in terms of their antioxidant activity. Hydrogen Peroxide (H2O2) activity leads to decrease of catalase and glutathione peroxidase activities. Selenium therapy reduces creatinine, urea and albuminuria levels as well as diabetic nephropathy edema results [28][29], and has a positive effect on diabetic wound healing. Selenium-enriched diet has not only antioxidative effect but is also involved in the protection of renal tubulointerstinum [30][29] and is effective at renal failure and end stage renal disease [31]– [33].

The GPX1 level in beta cells is small compared to other tissues, for example, its amount reaches only

1% compared to hepatocytes. Therefore, beta cells are very sensitive to the effects of hyperglycemia and inflammatory mediators (cytokines) [34]–[39]. Actually, problem of treating diabetes mellitus still remains relevant, since no experimental studies on diabetic rats using relatively high doses of selenium metallic nanoparticle suspension have been conducted. Implementation of the current study will allow to develop another effective, affordable and safe method of combined treatment for diabetes complication

## Methods

The research was conducted on the basis of A. Natishvili Institute of Morphology of Tbilisi State

University. Protocol of this research has been accepted bioethically.

Selenium nanoparticles were obtained by laboratory Nanomill (DECO-PBM-V-0.4L, China) and a high-frequency ultrasonic homogenizer - Ultrasonic Processor FS-1800N (China) [40]. Biochemical blood tests were performed using HumaLyzer Primus REF 18200 - semiautomatic photometer (Human Diagnostics, Germany) and a thermostat /incubator HUMACUBE (Human Diagnostics, Germany).

For conducting the morphological studies of animal organs, tissue sections were cut on the microtome

- Leica RM2235 (Leica Biosystems, USA), and observation was performed using a light microscope - Leica DM2500 (Leica Biosystems, USA).

Type 1 diabetes was induced in rats by using streptozotocin (STZ, Zanosar). The drug was purchased from Adooq BioScience LLC, USA) https://www.adooq.com/streptozotocin-zanosar.html. Monitoring of blood glucose levels was performed with a glucometer (One Touch Select, Switzerland). In the treatment which combined 10% selenium nanoparticles suspension with insulin, long-acting insulin (Novo Nordisk) was used. Administration was carried out by insulin syringe subcutaneously.

8-12-week-old albino rats were randomly divided into 4 groups: 10 rats per group (2 females and 8 males). Female rats were nulliparous and non-pregnant.

Type 1 diabetes was induced in rats by using streptozotocin (STZ, Zanosar). Diabetes mellitus was induced in rats according to the guidelines and pursuing our own modification (STZ 30 mg/kg). (Furman, B.L. 2015. Streptozotocin-induced diabetic models in mice and rats) [41]. After confirmation of diabetes mellitus, the rats were not administered any medications and/or studying suspension for 10 days; Administration of 10% selenium nanoparticles suspension or insulin was started only after 10 days and lasted for two weeks. Insulin was administered daily taking into account body weight and blood glucose levels. Rats had free access to both food and water throughout the experiment.

Caracterization of Study Groups

Group I - DM+Se - 10 rats with diabetes mellitus, taking only 10% selenium nanoparticles suspension. Group II - DM+Se+Ins- 10 rats with diabetes mellitus, taking 10% selenium nanoparticles suspension and insulin injection.

Group III - DM+Ins- rats with diabetes mellitus, receiving only insulin injection

Group IV- DM- rats with diabetes mellitus, not receiving any treatment

At the start of the experiment (10 days), blood glucose control was carried out every other day, while

after ten days, starting appropriate treatment, the blood glucose level was measured every day, based on the results obtained, insulin injection dose was determined.

After completion of the studies, rats had limited access to food for 24 hours (with free access to water)

and their euthanasia was performed using CO2, flow rate - 5 L/min in an individual euthanasia chamber/cage. Upon completion of the experiment, blood collection was performed via cardiac puncture. Blood tubes were centrifuged at 3000 rpm for 15 min. The obtained plasma was frozen(stored) in a refrigerator at -20°C. Subsequently, plasma was used to determine the blood biochemical parameters: Blood Urea Nitrogen (BUN), Creatinine (CREA), Albumin (ALB),

## Results and discussion

The biochemical studies conducted after mono- and combined treatment with 10% suspension of selenium nanoparticles in diabetic rats. (See Table 1)

| | CREA umol/L | BUN mmol/L | ALB g/L |
|---|---|---|---|
| Control Group | 31±4 | 4.6±1.7 | 42±4 |
| **DM + Se** | 30,2±13 | 5,3±5,2 | 36±2* |
| **DM+Se+Ins** | 29±11 | 4.5±3.2 | 44±3 |
| **DM+Ins** | 31±13 | 4,3±1,7 | 40±4 |
| **DM** | 64,3±5* | 9.6±0.8* | 28±4* |

*Table 1. Biochemical study of diabetic rats after mono and combined treatment with selenium nanoparticles.*
Mean±SD (n=5); *P<0.05 VS control group.
DM+Se diabetic rats treated with only 10% selenium nanoparticle suspension.
DM+Se+Ins Diabetic rats are treated with a combination of 10% selenium nanoparticle suspension and insulin injection.
DM+Ins diabetic rats are treated with insulin injections only.
DM diabetic rats do not receive any treatment

Morphological findings of Kidney obtained after mono- and combined treatment with 10% suspension of selenium nanoparticles and insulin in diabetic rats.

Group DM: Diabetic rats

Marked inflammatory infiltration and interstitial swelling were reflected. The renal glomerulus was wrinkled due to compression of the swollen renal interstitium (Fig. 1,2 A, B, C), "sludged" erythrocytes were visualized in the glomerular capillaries (Fig. 1C). Hemosiderin accumulation was visible in the ducts, the capillaries were dilated (Fig. 1 A, B, C). Protein components were seen in the interstitium of the kidney (Fig. 3).

Group DM+Ins: rats with diabetes mellitus, receiving only insulin injection

The renal tubules became swollen; the lumens are open. The lining epithelium was slightly swollen as well; the renal tubules are normal; no capillary stasis was revealed (Fig. 4).

Group DM+Se: rats with diabetes mellitus, taking only 10% selenium nanoparticle suspension

No inflammatory infiltration or necrosis, swelling of tubules or interstitium were expressed, renal glomeruli - Bowman's capsule was normal, tubular lumens were not dilated (Fig. 5), protein substances were not visible in the interstitium (Fig. 5, 6).

Group DM+Se+Ins: diabetic rats taking combined treatment of 10% selenium nanoparticle suspension and insulin Inflammatory infiltration or necrosis were not expressed in the kidneys. The structure of tubules and Bowman's capsule was preserved (Fig. 7). No swelling was observed in the kidneys by Fluorescence microscopy; protein substances were not accumulated in the interstitium (Fig. 8).

According to our study conducted in diabetic rats, the increase in creatinine (Table 1) coincided with an inflammatory infiltration in the kidneys - severe swelling of Bowman's capsules and collecting ducts, wrinkling of glomerulus due to the compression of swollen interstitium, sludged erythrocytes seen in the glomerular capillaries of the kidney, hemosiderin massive visualization in the ducts, dilated capillaries, expressed glomerulopathy (Fig. 1, 2, 3). Creatinine is known to be excreted via the kidneys along with urea. The urea cycle is one of the ways nitrogen is excreted from the body. The urea cycle describes the way how ammonia, alpha-nitrogen, aspartate and bicarbonate are converted into urea. Ammonia is toxic, while urea is relatively inert, soluble in water and readily excreted in the urine [42]– [46] As a result of the histological examination of the kidneys in diabetic rats, the changes discussed
above indicate increased serum urea caused by damage and dysfunction of kidney cells. Similar changes are indicating to the classic manifestation of diabetes mellitus [47]–[49] After mono and combined treatment with 10% selenium nanoparticles suspension and insulin, serum urea and creatinine levels were normalized (Table 1; P>0.05). Histological examination showed no swelling in renal tubulo- interstitium, the glomeruli and Bowman's capsule were normal, tubular lumens were not dilated, and no inflammatory infiltration was revealed (Fig. 7, 8). The obtained results are consistent with the literature data, where selenium/selenium nanoparticles showed profound effect in normalizing/reducing serum urine and creatinine levels and consequently, diabetic nephropathy that might be explained by reducing oxidative stress and inflammatory infiltration via intaking selenium nanoparticles [28]–[30], [50]–[53]

According to our study results, a decrease in albumin concentration was revealed in diabetic rats. Albumin is the most abundant protein found in plasma, determining its osmotic pressure, albumin synthesis takes place in hepatocytes, and thereby, the changes (decrease) in its amount can be regarded as hepatocyte damage indicators [54]. It should be noted that albumin has antioxidant properties and participates in the neutralization of free radicals, the level of which increases at various chronic diseases [55]–[59] Therefore, supposedly, albumin low levels, in addition to damage to hepatocytes, indicate its impaired ability to detoxify free radicals [55], [60]–[62]. It should be noted that decreasing the serum albumin level as a result of hepatocyte damage is considered as a typical biochemical change developed during diabetes mellitus [47], [51], [52], [63]–[66]. The reason for the decrease in serum albumin levels might be considered not only hepatocyte damage and thereby, decrease in its synthesis, but enhanced transition to

the tubulointestitium. Investigation of the kidney function and structure using fluorescence microscope clearly showed the particles of protein entering the kidney interstitium, corresponding to the impaired renal filtration function. Kidney diseases, including diabetic nephropathy is characterized by proteinuria and increased number of inflammatory mediators [67] – [70] thus damaging the basal membrane of renal tubules and moving protein into the peritubular space, in turn increasing tubulointerstinal damage [71]–[74] Decreased serum albumin levels in diabetic rats was seen. After treatment with a 10% selenium nanoparticles suspension mono and in combination with insulin therapy - albumin concentration increased during monotherapy and completely normalized with combined treatment with insulin and SeNPs (Table 1). This was confirmed by immunofluorescence microscopy data, showing a reduction in renal edema/swelling and absence of protein components in the renal interstitium (Fig. 7, 8).

## Conclusions

1. Monotherapy with a 10% selenium nanoparticles suspension compared with insulin monotherapy resulted in a reduction in renal edema in diabetic rats;
2. Combined treatment with a 10% selenium nanoparticles suspension and insulin prevents the development of kidney edema in diabetic rats;

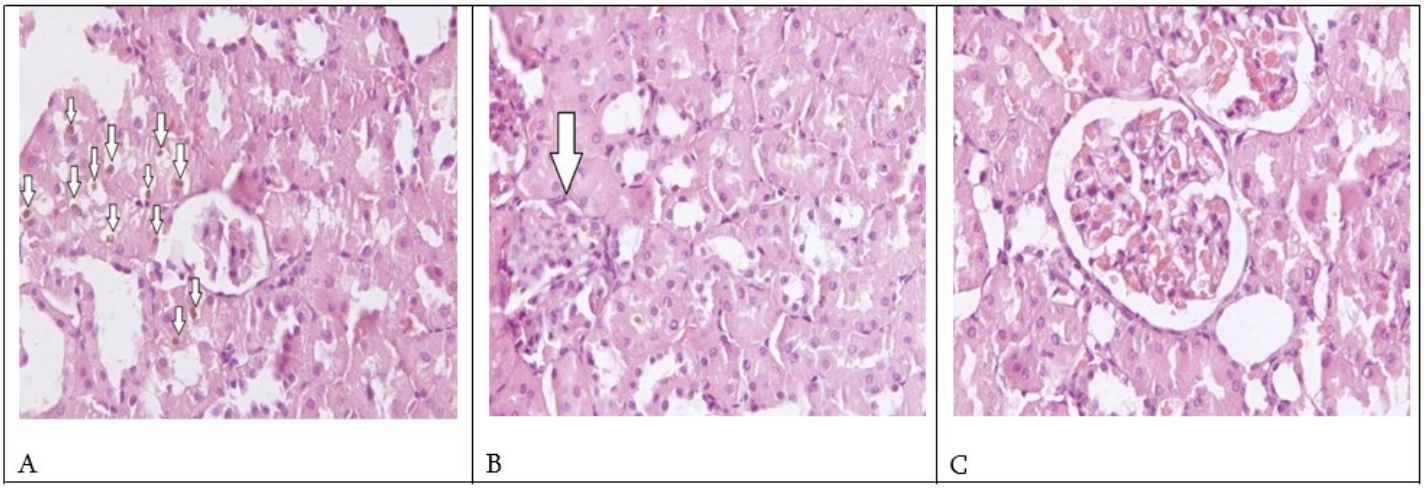


*Figure 1. DM rats, kidney. H&E. (X400). A: Renal ducts with large amounts of hemosiderin (arrow). B: Markedly wrinkled renal glomerulus (arrow), markedly dilated capillaries. C: Capillaries are dilated, glomeruli are constricted, erythrocyte stasis is seen.*

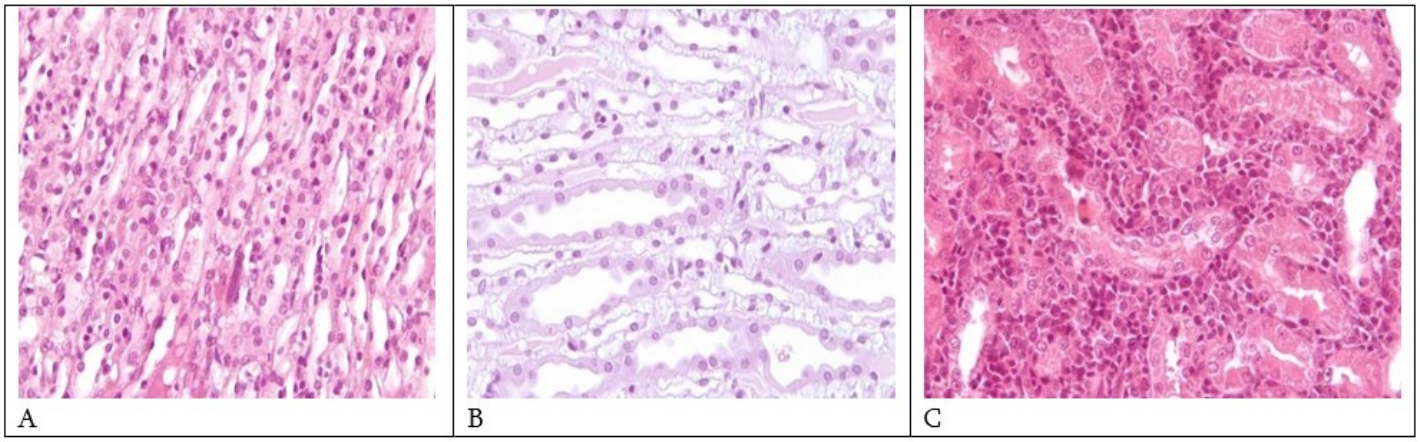


*Figure 2. DM rats, kidney. H&E. A: Collecting tubules are markedly swollen (X200). B: Interstitial edema is marked (X400). C: Renal cortex.*

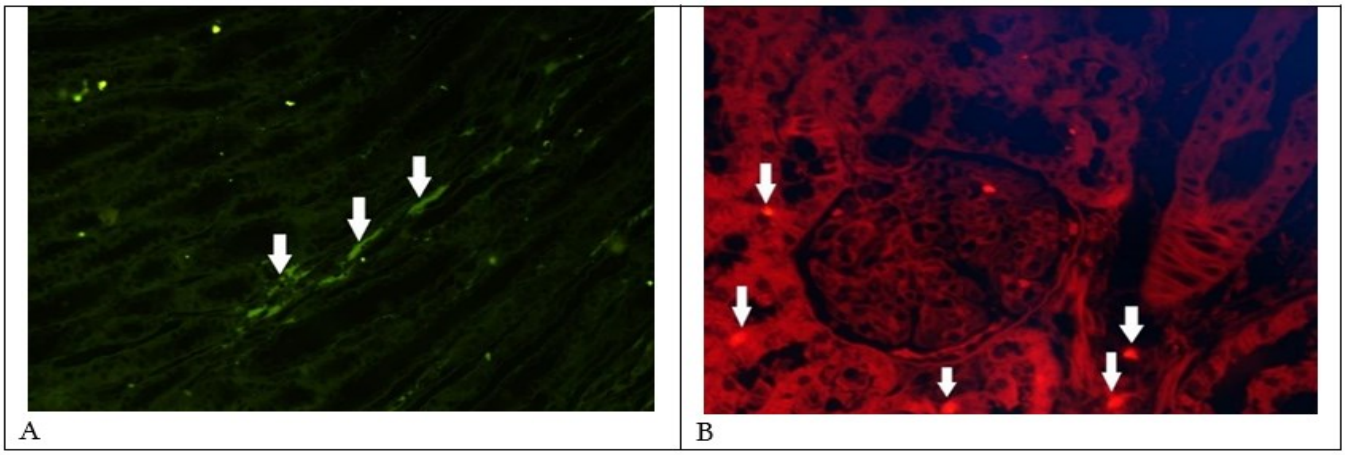


*Figure 3. DM rats, kidney (X400). Fluorescence microscope, protein accumulation in the interstitium (arrow)*

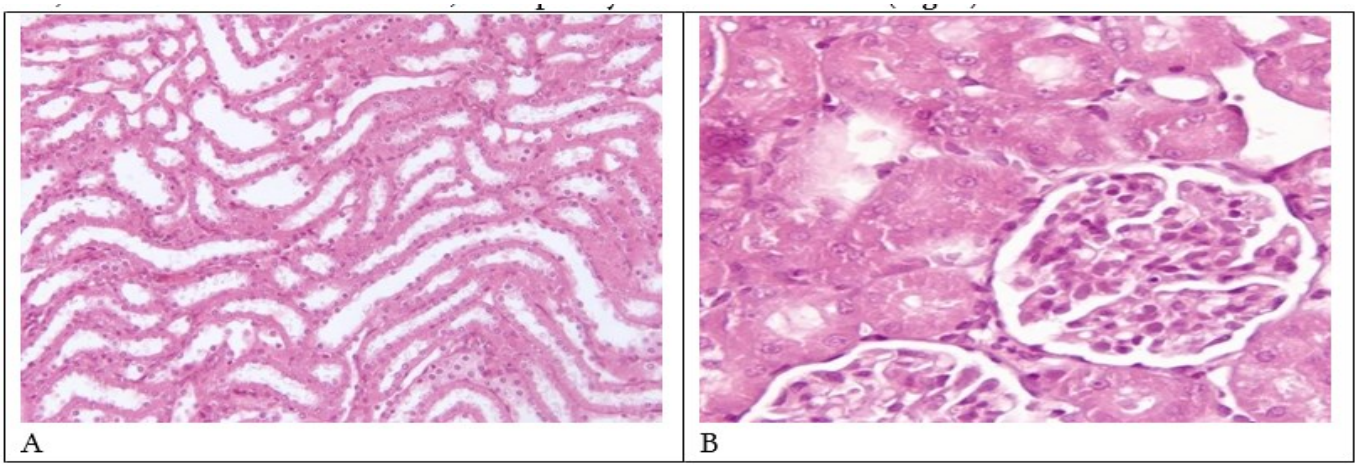


*Figure 4. Treatment of diabetic rats by injecting only insulin. Kidney H&E. A: (X200). The lining epithelium of the collecting ducts is slightly swollen B: (X400) Cortical substance of the kidney shows a normal Bowman's capsule, the lining epithelium of the proximal convoluted tubule is slightly swollen.*

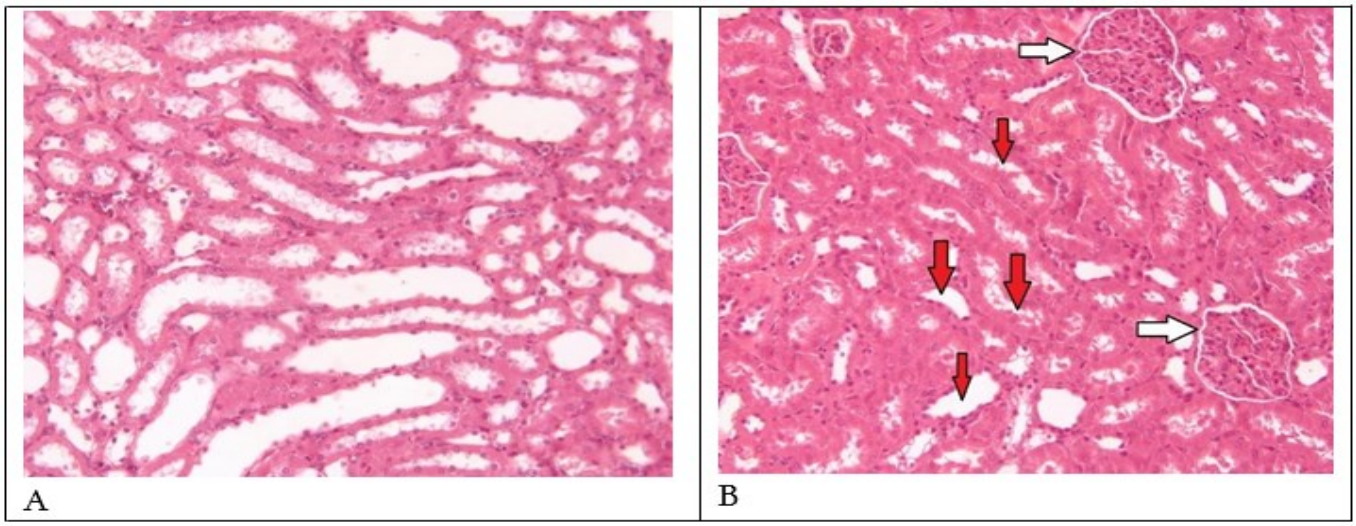


*Figure 5. DMrats, mono treatment with 10% SeNPs suspension, Kidneys. H&E. (X200). A: Renal cortex, proximal and distal convoluted tubules. Inflammatory infiltration, edema is not expressed. B: Renal glomeruli (white arrow) proximal and distal convoluted tubules (red arrow)*

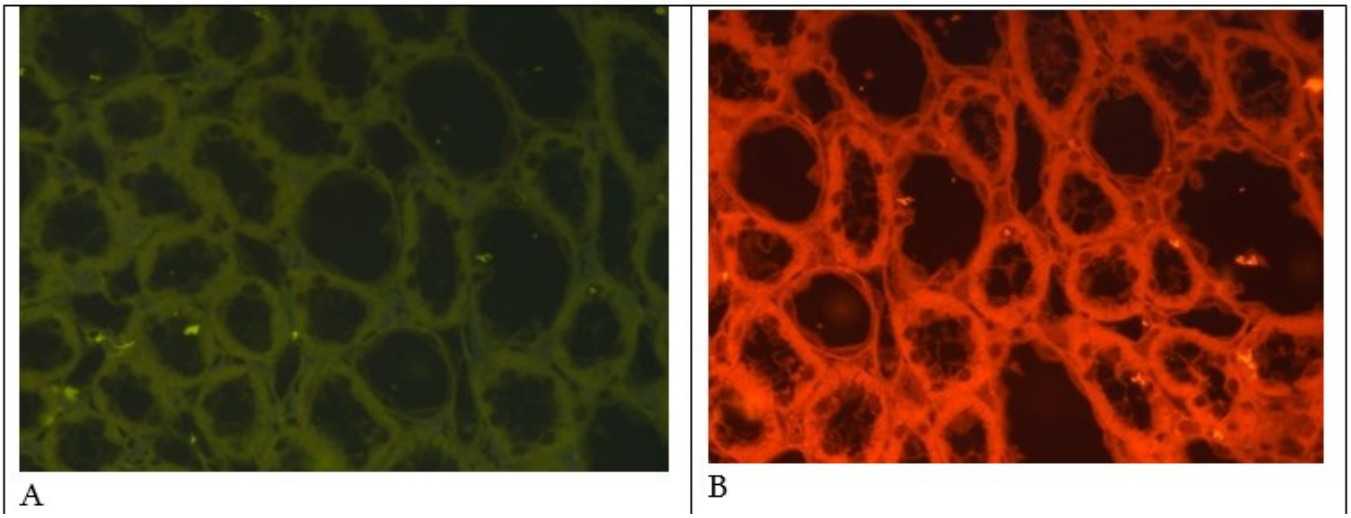


*Figure 6. DM rats, mono-treatment with 10% SeNPs suspension, kidneys. H&E. (X200) Fluorescence microscope shows that swelling is not seen in the kidneys, protein substances are not accumulated in the interstitium.*

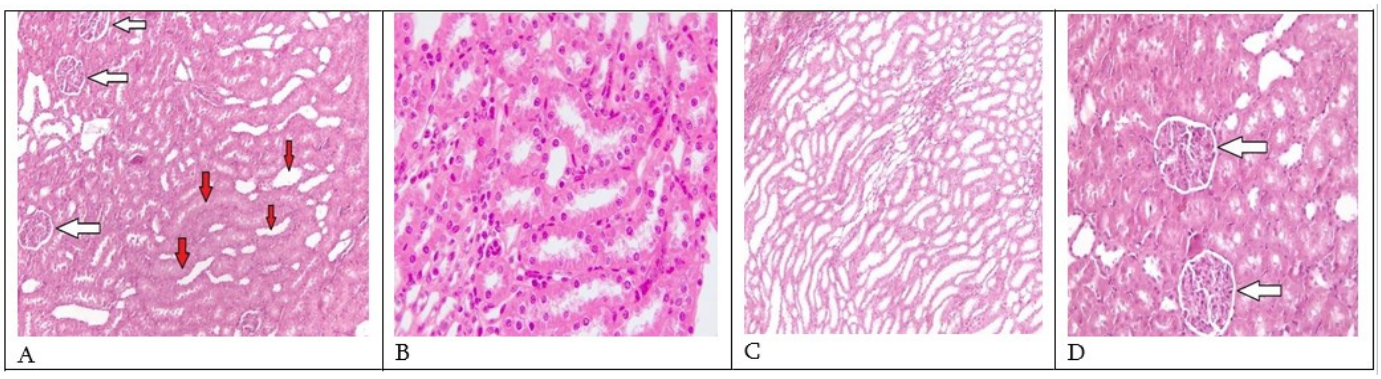


*Figure7. DM rats, combined treatment with 10% SeNPs suspension and insulin, kidney. H&E. A: (X200). Renal cortex, proximal and distal convoluted tubules (red arrow), renal glomerulus (white arrow), distal convoluted tubules slightly dilated. B: (X400) Normal proximal convoluted tubules. C: (X200) Normal renal collecting ducts, no marked edema or inflammatory infiltration. D: (X400) Normal Bowman's capsules (white arrow)*

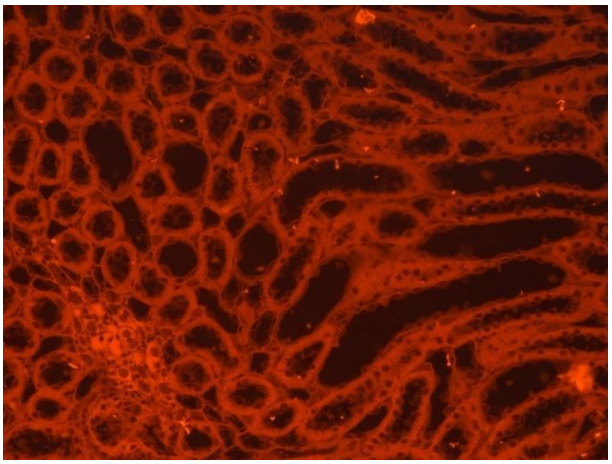

*Figure8. DM rats, combined treatment with 10% SeNPs suspension and insulin, kidney (X400). Can be seen by fluorescence microscope that there is no swelling in the kidneys, protein substances are not accumulated in the interstitium.*

## References


1. WHO, "Leading causes of death and disability. The top 10 causes of death," WHO reports, no. December, pp. 1–9, 2020, [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death#:~:text=In high-income countries%2C deaths,or 205000 deaths) respectively.
2. International Diabetes Federation, "IDF Diabetes Atlas 2021," 2021. [Online]. Available: https://diabetesatlas.org/atlas/tenth-edition/.
3. IDF, "Iternational Diabetes Federation DIABETES ATLAS Ninth edition 2019," 2019. [Online].Available:https://diabetesatlas.org/upload/resources/material/20200302_133351_IDFATLAS9e-final- web.pdf.
4. M. P. Rayman, "The importance of selenium to human health," Lancet, vol. 356, no. 9225, pp. 233–241, Jul. 2000, doi: 10.1016/S0140-6736(00)02490-9.
5. M. P. Rayman, "Selenium and human health," Lancet, vol. 379, no. 9822, pp. 1256–1268, 2012, doi: 10.1016/S0140-6736(11)61452-9.
6. R. Kvanchakhadze, Selenium in our world.pdf. 2018.
7. T. C. Stadtman, "Selenium Biochemistry," Annu. Rev. Biochem., vol. 59, no. 1, pp. 111–127, Jun. 1990, doi: 10.1146/annurev.bi.59.070190.000551.
8. S. A. P. Katrin Loeschner, Niels Hadrup, Marianne Hansen, H. R. L. Bente Gammelgaard, Laura Hyrup Møller, Alicja Mortensen, and and E. H. Larsen, "Absorption, distribution, metabolism and excretion of selenium following oral administration of elemental selenium nanoparticles or selenite in rats." Metallomics, 6(2), 330., p. 330, 2014, doi:10.1039/c3mt00309d.
9. A. Wadhwani & Utkarsha U. Shedbalkar & Richa Singh & Balu A. Chopade, "Biogenic selenium nanoparticles current status and future prospects," Appl. Microbiol. Biotechnol., no.100(6), pp. 2555–2566, 2016, doi: 10.1007/s00253-016-7300-7.
10. Z. Huang, B. J. Guo, R. N. S. Wong, and Y. Jiang, "Characterization and antioxidant activity of selenium-containing phycocyanin isolated from Spirulina platensis," Food Chem., vol. 100, no.3, pp. 1137–1143, 2007, doi: 10.1016/j.foodchem.2005.11.023.
11. M. H. Yazdi, M. Mahdavi, B. Varastehmoradi, M. A. Faramarzi, and A. R. Shahverdi, "The immunostimulatory effect of biogenic selenium nanoparticles on the 4T1 breast cancer model: An in vivo study," Biol. Trace Elem. Res., vol. 149, no. 1, pp. 22–28, 2012, doi: 10.1007/s12011-012-9402-0.
12. I. Wang, Yanbo Yan, Xuxia L Fu, "Effect of selenium nanoparticles with different sizes in primary cultured intestinal epithelial cells of crucian carp, Carassius auratus gibelio," Int. J. Nanomedicine, no. 2013:8, pp. 4007–4013, 2013, doi: 10.2147/IJN.S43691.
13. K. Anu, S. Devanesan, R. Prasanth, M. S. AlSalhi, S. Ajithkumar, and G. Singaravelu, "Biogenesis of selenium nanoparticles and their anti-leukemia activity," J. King Saud Univ. - Sci., vol. 32, no. 4, pp. 2520–2526, 2020, doi: 10.1016/j.jksus.2020.04.018.
14. W. Liu et al., "Selenium nanoparticles as a carrier of 5-fluorouracil to achieve anticancer synergism," ACS Nano, vol. 6, no. 8, pp. 6578–6591, 2012, doi: 10.1021/nn202452c.
15. L. D. Geoffrion et al., "Naked Selenium Nanoparticles for Antibacterial and Anticancer Treatments," ACS Omega, 2020, doi: 10.1021/acsomega.9b03172.
16. S. Wadhwani et al., "Green synthesis of selenium nanoparticles using Acinetobacter sp. SW30: optimization, characterization and its anticancer activity in breast cancer cells," Int. J. Nanomedicine, vol. Volume 12, pp. 6841–6855, Sep. 2017, doi: 10.2147/IJN.S139212.
17. H. Forootanfar et al., "Antioxidant and cytotoxic effect of biologically synthesized selenium nanoparticles in comparison to selenium dioxide," J. Trace Elem. Med. Biol., vol. 28, no. 1, pp.75–79, 2014, doi: 10.1016/j.jtemb.2013.07.005.
18. Q. Shen, B. Zhang, R. Xu, Y. Wang, X. Ding, and P. Li, "Antioxidant activity in vitro of the selenium-contained protein from the Se-enriched Bifidobacterium animalis 01," Anaerobe, vol. 16, no. 4, pp. 380–386, 2010, doi: 10.1016/j.anaerobe.2010.06.006.
19. V. Jayaprakash and J. R. Marshall, "Selenium and other antioxidants for chemoprevention of gastrointestinal cancers," Best Pract. Res. Clin. Gastroenterol., vol. 25, no. 4–5, pp. 507–518,2011, doi: 10.1016/j.bpg.2011.09.006.
20. C. Kornhauser, J. R. Garcia-Ramirez, K. Wrobel, E.-L. Pérez-Luque, M.-E. Garay-Sevilla, and K. Wrobel, "Serum selenium and glutathione peroxidase concentrations in type 2 diabetes mellitus patients," Prim. Care Diabetes, vol. 2, no. 2, pp. 81–85, Jun. 2008, doi:10.1016/j.pcd.2008.02.003.
21. H. Steinbrenner, E. Bilgic, L. Alili, H. Sies, and P. Brenneisen, "Selenoprotein P protects endothelial cells from oxidative damage by stimulation of glutathione peroxidase expression and activity," Free Radic. Res., vol. 40, no. 9, pp. 936–943, 2006, doi:10.1080/10715760600806248.
22. P. Brenneisen, H. Steinbrenner, and H. Sies, "Selenium, oxidative stress, and health aspects," Mol. Aspects Med., vol. 26, no. 4-5 SPEC. ISS., pp. 256–267, 2005, doi:10.1016/j.mam.2005.07.004.
23. M. B. Tabar, "Determination of serum selenium in patients with type II diabetes mellitus," Middle East J. Sci. Res., vol. 12, no. 4, pp. 433–435, 2012, doi:10.5829/idosi.mejsr.2012.12.4.2058.
24. G. Vassort and B. Turan, "Protective role of antioxidants in diabetes-induced cardiac dysfunction," Cardiovasc. Toxicol., vol. 10, no. 2, pp. 73–86, 2010, doi: 10.1007/s12012-010-9064-0.
25. B. A. Zachara et al., "The effect of selenium supplementation in the prevention of DNA damage in white blood cells of hemodialyzed patients: A pilot study," Biol. Trace Elem. Res., vol. 142, no. 3, pp. 274–283, 2011, doi: 10.1007/s12011-010-8776-0.
26. R. J. Shamberger, Biochemistry of Selenium, vol. 98, no. 5_Part_1. Boston, MA: Springer US,1983.
27. C. W. Lu, H. H. Chang, K. C. Yang, C. S. Kuo, L. T. Lee, and K. C. Huang, "High serum selenium levels are associated with increased risk for diabetes mellitus independent of centralobesity and insulin resistance," BMJ Open Diabetes Res. Care, vol. 4, no. 1, 2016, doi:10.1136/bmjdrc-2016-000253.
28. S. Roy, S. K. Dontamalla, A. K. Mondru, S. Sannigrahi, and P. R. Veerareddy, "Downregulation of apoptosis and modulation of TGF-β1 by sodium selenate prevents streptozotocin-induced diabetic rat renal impairment," Biol. Trace Elem. Res., vol. 139, no. 1, pp. 55–71, 2011, doi:10.1007/s12011-010-8635-z.
29. I. Hassan, H. Ebaid, J. Al-Tamimi, M. A. Habila, I. M. Alhazza, and A. M. Rady, "Selenium nanoparticles mitigate diabetic nephropathy and pancreatopathy in rat offspring via inhibition of oxidative stress," J. King Saud Univ. - Sci., vol. 33, no. 1, p. 101265, 2021, doi:10.1016/j.jksus.2020.101265.
30. A. S. Reddi and J. S. Bollineni, "Selenium-deficient diet induces renal oxidative stress and injury via TGF-β1 in normal and diabetic rats," Kidney Int., vol. 59, no. 4, pp. 1342–1353,2001, doi: 10.1046/j.1523-1755.2001.0590041342.x.
31. B. A. Zachara, Selenium and selenium-dependent antioxidants in chronic kidney disease, 1st ed., vol. 68. Elsevier Inc., 2015.
32. I. Kiss, "Importance of selenium homeostasis in chronic and end-stage kidney diseases," Orv.Hetil., vol. 154, no. 41, pp. 1641–1647, Oct. 2013, doi: 10.1556/OH.2013.29733.
33. P. Iglesias, R. Selgas, S. Romero, and J. J. Díez, "Selenium and kidney disease," J. Nephrol., vol.26, no. 2, pp. 266–272, 2013, doi: 10.5301/jn.5000213.
34. X. G. Lei and M. Z. Vatamaniuk, "Two tales of antioxidant enzymes on β cells and diabetes," Antioxidants Redox Signal., vol. 14, no. 3, pp. 489–503, 2011, doi: 10.1089/ars.2010.3416.
35. J. Wang and H. Wang, "Oxidative Stress in Pancreatic Beta Cell Regeneration," Oxid. Med. Cell. Longev., vol. 2017, pp. 1–9, 2017, doi: 10.1155/2017/1930261.
36. S. Harmon et al., "β-cell-specific overexpression of glutathione peroxidase preserves intranuclear MafA and reverses diabetes in db/db Mice," Endocrinology, vol. 150, no. 11, pp.4855–4862, 2009, doi: 10.1210/en.2009-0708.
37. S. Lenzen, J. Drinkgern, and M. Tiedge, "Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues," Free Radic. Biol. Med., vol. 20, no. 3, pp. 463–466, 1996, doi: 10.1016/0891-5849(96)02051-5.
38. L. A. Sigfrid et al., "Antioxidant enzyme activity and mRNA expression in the islets of Langerhans from the BB/S rat model of type 1 diabetes and an insulin-producing cell line," J. Mol. Med., vol. 82, no. 5, pp. 325–335, 2004, doi: 10.1007/s00109-004-0533-4.
39. J. D. Acharya and S. S. Ghaskadbi, "Islets and their antioxidant defense," Islets, vol. 2, no. 4, pp. 225–235, Jul. 2010, doi: 10.4161/isl.2.4.12219.
40. G. M. Salome Khubulava, Nino Chichiveishvili, Nodar Khodeli, Giorgi Phichkhaia, "Preparation of Selenium Nanoparticles with Mechano-sonochemical Methods," Asian J. Pharm., vol. 12, no. 02, 2018, doi: 10.22377/ajp.v12i02.2405
41. B. L. Furman, "Streptozotocin-Induced Diabetic Models in Mice and Rats," Curr. Protoc.Pharmacol., vol. 70, no. 1, pp. 5.47.1-5.47.20, Sep. 2015, doi: 10.1002/0471141755.ph0547s70.
42. N. A. Mew, M. B. Pappa, and A. L. Gropman, Urea Cycle Disorders, Fifth Edit. Elsevier Inc.,2014.
43. F. Rossignol, N. Ah Mew, M. R. Meltzer, and A. L. Gropman, "Urea cycle disorders," in Rosen berg's Molecular and Genetic Basis of Neurological and Psychiatric Disease, Elsevier,2020, pp. 827–848.
44. E. G. G. Yvonna S. Lincoln, Naturalistic Inquiry. Sage, 1985.
45. G. Litwack, "Metabolism of Amino Acids," in Human Biochemistry, Elsevier, 2018, pp. 359–394.
46. A. Kumari, "Urea Synthesis," in Sweet Biochemistry, Elsevier, 2018, pp. 41–44.
47. G. S. Kumar, A. Kulkarni, A. Khurana, J. Kaur, and K. Tikoo, "Selenium nanoparticles involve HSP-70 and SIRT1 in preventing the progression of type 1 diabetic nephropathy," Chem. Biol. Interact., vol. 223, pp. 125–133, 2014, doi: 10.1016/j.cbi.2014.09.017.
48. H. Elbe, N. Vardi, M. Esrefoglu, B. Ates, S. Yologlu, and C. Taskapan, "Amelioration of streptozotocin-induced diabetic nephropathy by melatonin, quercetin, and resveratrol in rats," Hum. Exp. Toxicol., vol. 34, no. 1, pp. 100–113, 2015, doi: 10.1177/0960327114531995.
49. S. N. Mestry, J. B. Dhodi, S. B. Kumbhar, and A. R. Juvekar, "Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by Punica granatum Linn. leaves extract," J. Tradit. Complement. Med., vol. 7, no. 3, pp. 273–280, 2017, doi: 10.1016/j.jtcme.2016.06.008.
50. M. Rezaei-Kelishadi et al., "Effects of selenium nanoparticles on kidney and liver functional disorders in streptozotocin-induced diabetic rats," Physiol. Pharmacol., vol. 21, no. 2, pp. 155–162, 2017.
51. S. Khater, I. Ali, S. Khater, A. Ahmed, and S. abd el-megid, "Preparation and Characterization of Chitosan-Stabilized Selenium Nanoparticles for Ameliorating Experimentally Induced Diabetic Nephropathy in Rats," Arab J. Nucl. Sci. Appl., vol. 0, no. 0, pp. 1–9, Jun. 2020, doi:10.21608/ajnsa.2020.19809.1300.
52. S. A. Abdulmalek and M. Balbaa, "Synergistic effect of nano-selenium and metformin on type2 diabetic rat model: Diabetic complications alleviation through insulin sensitivity, oxidative mediators and inflammatory markers," PLoS One, vol. 14, no. 8,

p. e0220779, Aug. 2019, doi:10.1371/journal.pone.0220779.
53. A. Khurana, S. Tekula, M. A. Saifi, P. Venkatesh, and C. Godugu, "Therapeutic applications of selenium nanoparticles," Biomed. Pharmacother., vol. 111, no. December 2018, pp. 802–812, Mar. 2019, doi: 10.1016/j.biopha.2018.12.146.
54. L. Sun et al., "Impaired albumin function: a novel potential indicator for liver function damage?," Ann. Med., vol. 51, no. 7–8, pp. 333–344, 2019, doi:10.1080/07853890.2019.1693056.[55] J. P. Nicholson, M. R. Wolmarans, and G. R. Park, "REVIEW ARTICLE The role of albumin in critical illness," Br. J. Anaesth., vol. 85, no. 4, pp. 599–610, 2000.
55. M. Taverna, A.-L. Marie, J.-P. Mira, and B. Guidet, "Specific antioxidant properties of human serum albumin," Ann. Intensive Care, vol. 3, no. 1, p. 4, 2013, doi: 10.1186/2110-5820-3-4.
56. M. Roche, P. Rondeau, N. R. Singh, E. Tarnus, and E. Bourdon, "The antioxidant properties of serum albumin," FEBS Lett., vol. 582, no. 13, pp. 1783–1787, Jun. 2008, doi:10.1016/j.febslet.2008.04.057.
57. M. E. Holt, M. E. T. Ryall, and A. K. Campbell, "Albumin inhibits human polymorphonuclear leucocyte luminol-dependent chemiluminescence: Evidence for oxygen radical scavenging," Br. J. Exp. Pathol., vol. 65, no. 2, pp. 231–241, 1984.
58. T. Ahn, C. S. Bae, and C. H. Yun, "Selenium supplementation restores the decreased albumin level of peripheral blood mononuclear cells in streptozotocin-induced diabetic mice," J. Vet. Med. Sci., vol. 78, no. 4, pp. 669–674, 2016, doi: 10.1292/jvms.15-0611.
59. M. Sitar, S. Aydin, and U. ÇAkatay, "Human Serum Albumin and Its Relation with Oxidative Stress," Clin. Lab., vol. 59, no. 09+10/2013, pp. 945–952, 2013, doi: 10.7754/Clin.Lab.2012.121115.
60. S. Prakash, "Role of Human Serum Albumin and Oxidative Stress in Diabetes," J. Appl. Biotechnol. Bioeng., vol. 3, no. 1, pp. 281–285, May 2017, doi: 10.15406/jabb.2017.03.00057.
61. J. Himmelfarb and E. McMonagle, "Albumin is the major plasma protein target of oxidant stress in uremia," Kidney Int., vol. 60, no. 1, pp. 358–363, 2001, doi: 10.1046/j.1523-1755.2001.00807.x.
62. T. M. Osicka et al., "Prevention of albuminuria by aminoguanidine or ramipril in streptozotocin-induced diabetic rats is associated with the normalization of glomerular protein kinase C," Diabetes, vol. 49, no. 1, pp. 87–93, 2000, doi: 10.2337/diabetes.49.1.87.
63. K. T. Park, C. H. Yun, C. S. Bae, and T. Ahn, "Decreased level of albumin in peripheral blood mononuclear cells of streptozotocin-induced diabetic rats," J. Vet. Med. Sci., vol. 76, no. 8, pp.1087–1092, 2014, doi: 10.1292/jvms.13-0631.
64. E. N. Sundaram, P. U. M. Reddy, and K. P. Singh, "Effect of alcoholic extracts of Indian medicinal plants on the altered enzymatic activities of diabetic rats," Indian J. Pharm. Sci., vol.71, no. 5, pp. 594–598, 2009, doi: 10.4103/0250-474X.58175.
65. T. O. Sunmonu and A. J. Afolayan, "Evaluation of Antidiabetic Activity and Associated Toxicity of Artemisia afra Aqueous Extract in Wistar Rats," Evidence-Based Complement. Altern. Med., vol. 2013, pp. 1–8, 2013, doi: 10.1155/2013/929074.
66. J. L. Gross, M. J. de Azevedo, S. P. Silveiro, L. H. Canani, M. L. Caramori, and T. Zelmanovitz, "Diabetic Nephropathy: Diagnosis, Prevention, and Treatment," Diabetes Care, vol. 28, no. 1, pp. 164–176, Jan. 2005, doi: 10.2337/diacare.28.1.164.
67. C. D. A. Stehouwer, M. Gall, J. W. R. Twisk, E. Knudsen, J. J. Emeis, and H.-H. Parving,"Increased Urinary Albumin Excretion, Endothelial Dysfunction, and Chronic Low-Grade Inflammation in Type 2 Diabetes: Progressive, Interrelated, and Independently Associated With Risk of Death," Diabetes, vol. 51, no. 4, pp. 1157–1165, Apr. 2002, doi:10.2337/diabetes.51.4.1157.
68. J. A. Jefferson, S. J. Shankland, and R. H. Pichler, "Proteinuria in diabetic kidney disease: Amechanistic viewpoint," Kidney Int., vol. 74, no. 1, pp. 22–36, Jul. 2008, doi:10.1038/ki.2008.128.
69. A. K. H. Lim and G. H. Tesch, "Inflammation in diabetic nephropathy," Mediators Inflamm., vol. 2012, 2012, doi: 10.1155/2012/146154.
70. A. A. Eddy, "Proteinuria and interstitial injury," Nephrol. Dial. Transplant., vol. 19, no. 2, pp.277–281, Feb. 2004, doi: 10.1093/ndt/gfg533.
71. N. S. SHEERIN and S. H. SACKS, "Leaked protein and interstitial damage in the kidney: is complement the missing link?," Clin. Exp. Immunol., vol. 130, no. 1, pp. 1–3, Oct. 2002, doi:10.1046/j.1365-2249.2002.01979.x.
72. M. Zeisberg and R. Kalluri, "Physiology of the Renal Interstitium," Clin. J. Am. Soc. Nephrol., vol. 10, no. 10, pp. 1831–1840, Oct. 2015, doi: 10.2215/CJN.00640114.
73. E. I. Christensen and J. Gburek, "Protein reabsorption in renal proximal tubule? function and dysfunction in kidney pathophysiology," Pediatr. Nephrol., vol. 19, no. 7, pp. 714–721, Jul. 2004, doi: 10.1007/s00467-004-1494-0.