

Original Article

Impact of Nanochitosan Alcoholic Basil Seed Extract on Albino Diabetic Rats

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**How to Cite This Article** Al khazaali, I. T. H., & Zeiny, S. S. M. (2025). Impact of Nanochitosan Alcoholic Basil Seed Extract on Albino Diabetic Rats. *Iranian Journal of Veterinary Medicine*, 19(4), 705-712. <http://dx.doi.org/10.32598/ijvm.19.4.1005617> <http://dx.doi.org/10.32598/ijvm.19.4.1005617>**ABSTRACT**

Background: Basil seeds are traditionally used for various therapeutic purposes, such as improving digestion, regulating blood sugar, aiding weight loss, cooling the body, relieving stress, lowering blood pressure and cholesterol, improving vision, and reducing inflammation. Recent studies show that *Ocimum basilicum* extracts have hypoglycemic and hypolipidemic effects in rats. Due to diabetes' significant impact on the cardiovascular system, researchers are exploring natural treatments, including plant-based remedies. Nanomaterials are emerging as a promising method for effectively delivering these herbal treatments.

Objectives: This study aims to investigate the effect of nanoparticle chitosan of basil seed alcohol extract on albino rats with type 1 diabetes mellitus.

Methods: Basil seed alcoholic extract nanoparticles were examined using Fourier transform infrared spectroscopy, x-ray powder diffraction and field-emission scanning electron microscope. A total of 42 adult male rats were divided into six groups: One negative control, one positive control (diabetes induced with alloxan at 100 mg/kg) and four treatment groups. The treatments were empagliflozin (25 mg/kg), nanochitosan (250 mg/kg), basil seed alcohol extract (250 mg/kg) and nanoparticle basil seed extract (250 mg/kg), administered orally for 21 days. Blood samples were collected to evaluate sugar levels, interleukin-1 β , tumor necrosis factor- α and insulin.

Results: The study investigated the effects of nanochitosan and basil extract in diabetic rats. Diabetes was induced in five groups of rats, leading to increased blood glucose levels. Key findings included elevated insulin levels in all treated groups except the positive control group, and the nano-chitosan basil seed extract group showed the most significant improvement. Interleukin-1 β level decreased in all treated groups, with significant reductions observed in groups 5 and 6. Similarly, Tumor necrosis factor level decreased in all treated groups, with the nanochitosan basil seed extract group showing the most significant reduction compared to groups 3, 4 and 5. The nanochitosan basil seed extract demonstrated substantial positive effects on insulin, interleukin-1 β and tumor necrosis factor- α levels in diabetic rats.

Conclusion: Using basil seed extract combined with nanochitosan has shown a positive impact on increasing insulin levels, reducing blood sugar, and decreasing interleukin-1 β and tumor necrosis factor- α levels, which are associated with inflammation and chronic diseases.

Keywords: Diabetes, Nano *Ocimum basilicum* seed extract, Rats

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Introduction

Basil seeds are traditionally used for therapeutic purposes to improve digestive health, regulate blood sugar, aid in weight loss efforts, cool the body, relieve stress, lower blood pressure, improve vision, lower cholesterol and reduce inflammation. Recently, the hypoglycemic and hypolipidemic effects of extracts of various parts of *Ocimum sanctum* and *Ocimum basilicum* were studied on rats (Cherian, 2019).

Secondary metabolites of *Ocimum* species possess exceptional biological activities. They show bactericide, fungicide, repellent, anti-inflammatory, antioxidative, antidiarrheic, chemopreventive and radioprotective effects (Alabedi et al., 2021; Hamzah et al., 2025).

Diabetes, a widespread health concern, can affect heart and blood vessels. Researchers are exploring alternative treatments using natural materials like plants. One exciting area involves nanomaterials that can help deliver herbal remedies.

Material and Methods

Treatment preparation

Basil seed extract: The seeds bought from the market were ground into a fine powder. 100 g of this powder were macerated with 1000 mL of 70% methanol and stirred magnetically for 24 hours at 45 °C. The resulting extract was filtered first through gauze, then through No. 1 filter paper. It was concentrated using a rotary evaporator (90 rpm) at 40 °C and under reduced pressure. The concentrated extracts were stored in a refrigerated sample container (Harbone, 1988).

Chitosan nanoparticles (CNPs): A chitosan solution is prepared using a modulating method. The solution, at a concentration of 4 mg/mL, is produced by dissolving 200 mg of chitosan powder in 50 mL of deionized distilled water containing 1% acetic acid. This mixture was kept at room temperature for 24 hours. It was then stirred continuously with a magnetic bar on a hotplate stirrer at 900 rpm for 30 minutes, creating a semi-colloidal solution. The pH of the solution was adjusted to 4.6, the optimal level for chitosan surface adsorption, using a pH meter and adding NaOH (0.1 N) (Patil & Pandey, 2017).

In the study, nanoparticles were analyzed using several advanced techniques. Fourier-transform infrared spec-

troscopy (FTIR) was employed to identify the chemical bonds within molecules, providing unique molecular signatures. X-ray diffraction (XRD) was used to determine the composition and crystal structure of the nanoparticles, offering precise measurements of atomic distances and crystalline grain size. Additionally, scanning electron microscopy (SEM) was utilized to examine the nanoparticles' size, shape and surface morphology through direct visualization, offering detailed morphological insights.

Finally, CNPs were prepared suitable for drug loading and various applications. Subsequently, the samples were centrifuged at 900 rpm for 15 minutes to obtain serum. The nanochitosan was then administered by dissolving 250 g in distilled water, with a daily volume dose of 0.1 mL/100 g of rat body weight (Tripathy et al., 2012; Hamzah et al., 2024).

Loading basil seed extract with CNPs: To extract and synthesize CNPs, 10 mL of *O. basilicum* seed extract was added to 10 mL of a chitosan solution. This mixture was magnetically stirred (110 rpm) at 60 °C to form an opalescent solution (Metwaly et al., 2023). Next, the nanochitosan basil seed solution was prepared to administer a dose of 250 mg/kg per day, with 0.1 mL for every 100 g of rat body weight. Empagliflozin was then prepared by dissolving 25 g of the extract in 1 mL of distilled water, administering 0.1 mL for every 100 g of body weight.

Study animals

A total of 42 adult male rats, each weighing between 225 and 275 g, were used. The experimental procedures began after a two-week acclimatization period. The animals were randomly divided into different cages, each housing seven animals.

Induction of diabetes

Thirty-five experimental rats were induced with type I diabetes mellitus. They were subjected to a 24-hour fasting period and administered 100 mg/kg of alloxan to each rat. After 6 hours, the animals received a 5% sucrose solution. The blood sugar level of each rat was examined after three days using a glucometer.

Experimental animals

There were 7 healthy rats as the control negative group who received distilled water. A total of 35 rats with type 1 diabetic mellitus were divided into five groups (7 rats in each group) for treatment as follows:

Positive control group: This group received a single dose of alloxan once (100 mg/kg intraperitoneally [IP]).

Basil alcohol extract group: This group received a single dose of alloxan (100 mg/kg IP) plus *O. basilicum* seed alcohol extract at 250 mg/kg orally every day.

Nanochitosan group: This group received a single dose of alloxan (100 mg/kg IP) plus nanochitosan at 250 mg/kg orally daily.

Nanochitosan-basil seed extract group: This group received a single dose of alloxan (100 mg/kg IP) plus nanochitosan basil at 250 mg/kg orally every day.

Empagliflozin group (n=7): This group received a single dose of alloxan (100 mg/kg IP) plus empagliflozin at 25 mg/kg orally daily.

Groups 3, 4, 5 and 6 were treated for 21 days. At the end of the experiment, all animals were prepared to collect blood samples by heart puncture using a 23-gauge needle syringe for further assessment and analysis.

The blood was drawn from all animals and allowed to rest for 15 minutes and then centrifuged to obtain serum. The animals were anesthetized using intramuscular injection of ketamine (90 mg/kg body weight) and xylazine (40 mg/kg body weight) for blood collected from the animals at the end of the experiment by gel tub (Bustani et al., 2024; Bustani & Alghetaa, 2024). The serum was centrifuged at 900 rpm for 10 minutes.

Serum tests

Measuring interleukin-1 β using ELISA

The analysis utilized a specialized interleukin-1 β (IL-1 β) kit (Melsin Company, China). The content assay kit (Cat No.: EKRAT-0419) was designed to measure IL-1 β in various samples quantitatively.

Measuring tumor necrosis factor- α using ELISA (similar to IL-1 β)

The analysis utilized a specialized necrotic factor kit (Melsin Company, China). The content assay kit (Cat No.: EKRAT-0419) was designed to quantitatively measure tumor necrosis factor (TNF- α) in samples.

Measuring insulin using wet chemistry analyzers

Insulin was tested using a kit from Nalondi, Iran, and a UV-spectrophotometer (VS721G brand) from the UK was employed.

Statistical analysis

The collected data underwent statistical analysis using GraphPad Prism software, version 9, which applied the on-way analysis of variance (ANOVA). This analysis incorporated the Holm-Sidak correction for multiple comparisons, with a significance threshold set at $P < 0.05$.

Results

The results of basil seed extract nanoparticles were positive according to the tests.

FTIR analysis

The FTIR analysis of CNPs and basil seeds composite (CNPs/OBE) demonstrated characteristic peaks. The CNPs exhibited key peaks at 1100 cm^{-1} (C–O stretch), 1400 cm^{-1} (bridge O stretch), 1656 and 1620 cm^{-1} (N–H bend), 2900 cm^{-1} (C–H stretch), and 3500 cm^{-1} (–OH stretch). The basil seeds' extract showed strong peaks at 2950 and 2850 cm^{-1} (vibrational stretching of –CH₂ groups) and 1650 cm^{-1} (carboxylate anions), indicating intermolecular hydrogen bonding between the basil extract and chitosan.

SEM analysis

SEM revealed the morphology of CNPs and the CNPs-basil seed composite. The CNPs exhibited a globular shape, while the basil seeds extract showed a bed and chain-like structure. The composite nanoparticles had an average particle size of 43.92 nm.

XRD analysis

XRD showed distinct crystalline peaks for the CNPs and the basil seed extract. The XRD pattern for the composite CNPs-basil seed extract displayed peaks at $2\theta = 20^\circ$, 24° and other characteristic points, indicating a high degree of crystallinity in the composite material (Suhail et al., 2020).

Insulin concentration

In Figure 1, the assessment results of insulin concentration are displayed. The negative control group showed no significant difference compared to the groups 4 and

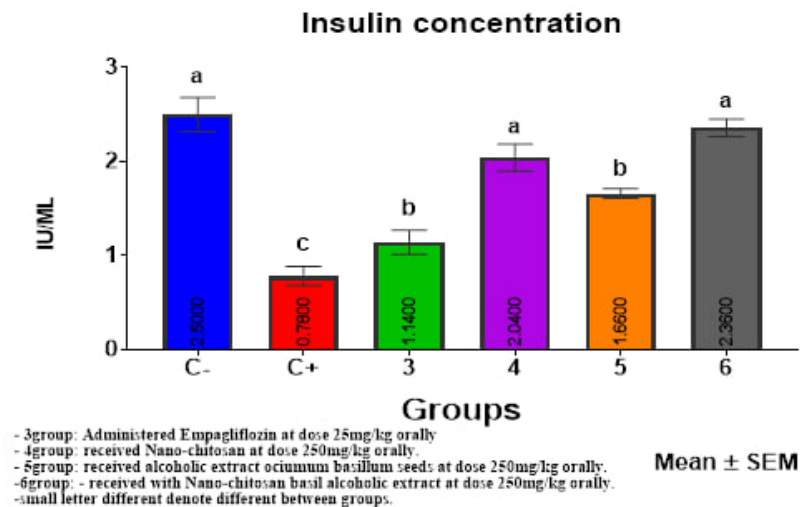


Figure 1. Assessment of insulin concentration across different experimental groups

6. However, there was a highly significant increase in insulin concentration in the positive control group and groups 3 and 5 compared to the negative control group ($P < 0.05$). There was no significant difference between groups 3 and 5, nor between groups 4 and 5, but a highly significant difference was observed when compared with the positive control group ($P < 0.05$).

Interleukin-1 β

Figure 2 displays the assessment results of interleukin-1 β . The control positive showed an increased level of IL-1 β compared with the treated groups and negative

control and showed no significant difference compared to groups 4 and 3. However, there was a statistically significant difference compared to groups 5 and 6 ($P < 0.05$). While control negative changes appeared to be not significant compared to groups 5 and 6.

Tumor necrotic factor

In Figure 3, the assessment results of the TNF- α are displayed. The positive control group showed a statistically significant high TNF- α compared to all other groups. But no significant difference between the negative control group and other treated groups.

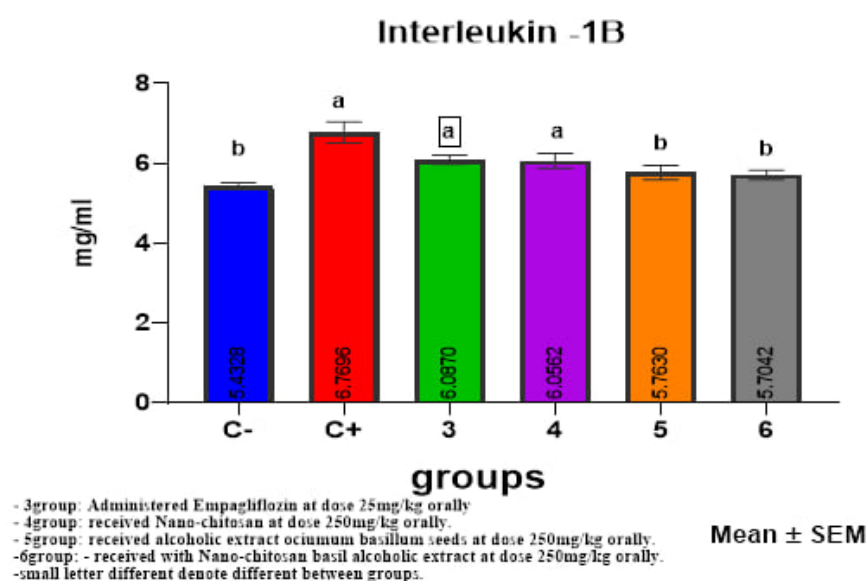


Figure 2. Assessment of interleukin-1 β levels across different experimental groups

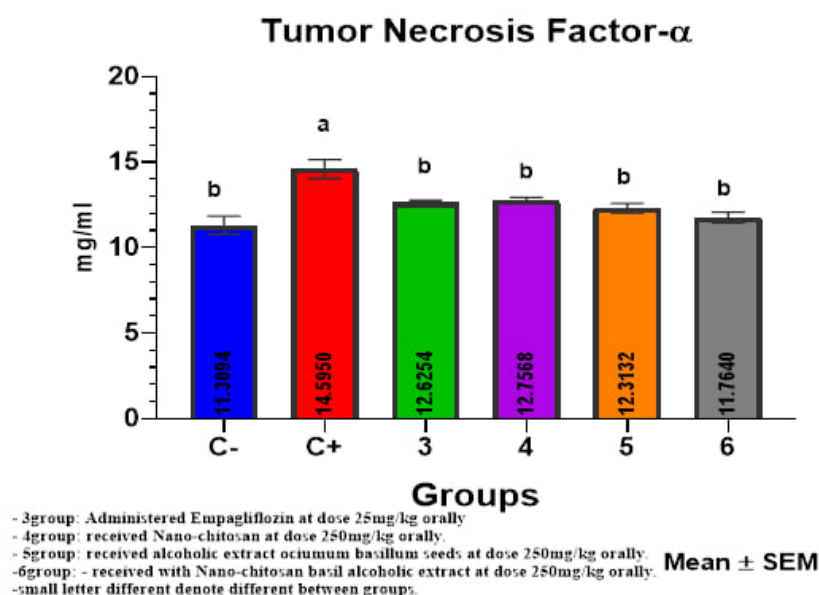


Figure 3. Assessment of TNF- α levels across different experimental groups

Discussion

Insulin concentration

The results of insulin concentration indicated that its secretion decreased from β cells because the impact of alloxan was evident in the destruction of beta cells, leading to reduced insulin secretion and elevated blood sugar levels in diabetic rats (Radenković et al., 2016; Adeleye et al., 2024).

Alloxan, a toxic glucose analog, accumulates selectively in pancreatic beta cells. It induces the generation of reactive oxygen species through redox reactions, leading to beta cell death. Alloxan also hampers glucose-induced insulin secretion by interfering with the beta cell glucose sensor (Cheraghi, 2024; Moradifar et al., 2024).

After treating the animals, an increase in insulin secretion appeared in the nanochitosan and nanochitosan-basil groups more than in empagliflozin and hydroalcoholic basil extract groups because the basil nanochitosan combines the effects of both basil seeds extract and nanochitosan (Salih et al., 2019a; Salih et al., 2019b). The combined effect of these two substances could be responsible for the insulin concentration observed in this group after three weeks. Regarding the nanochitosan a study has shown that nanochitosan can increase glucose absorption but did not significantly affect insulin levels. Insulin secretion capacity was enhanced by COS feeding regardless of alloxan treatment, even though statistical significance was not observed. These findings imply

that chitosan could boost insulin production in pancreatic β -cells and regulate plasma glucose metabolism, as suggested by Liu et al. (2010).

The influence of alcoholic basil extract to increase insulin levels is attributed to the enhanced functionality of the pancreas's β -cells as an herbal medicine for diabetic disease.

Empagliflozin appears to increase insulin secretion less than other treatment groups. According to Al Jobori et al. (2018), empagliflozin treatment in mice increased β -cell glucose sensitivity and enhanced β -cell function, as measured by the insulin secretion/insulin resistance index. This finding agrees with the study by Cheng et al. (2016), which also reported increased insulin mRNA expression in empagliflozin-treated mice.

TNF- α and IL-1 β concentrations

Another crucial aspect to consider is the significant increase in levels of pro-inflammatory cytokines, such as TNF- α and IL-1 β , observed in diabetic rats. We note from the research results for TNF- α and IL-1 β that they increased in the control positive group and began to decrease in the treatment groups. Similar results formerly reported by Othman et al. (2021) were in experimentally induced diabetes. The levels of these cytokines rise due to excess free radicals, which amplify the nuclear factor-kappa-B (NF- κ B). This factor is crucial in transcribing genes that encode inflammatory proteins. The link between oxidative stress and TNF- α expression is attribut-

ed to the activation of NF- κ B and p38 mitogen-activated protein kinase by reactive oxygen species, resulting in further release of TNF- α (Cheraghi et al., 2021; Sead et al., 2023; Satarzadeh et al., 2024).

However, using a hydroalcoholic extract from basil effectively reduced these cytokine levels in diabetic rats, indicating its preventive measure against inflammation in diabetes. This finding is corroborated by earlier studies such as Claudino et al. (2007), including the anti-inflammatory effects of *O. basilicum* (basil) against acute and chronic inflammation models.

In addition, the extract of *O. basilicum* protects against acute inflammation caused by alloxan by inhibiting the release of pro-inflammatory cytokines and lipid peroxidative damage (Claudino et al., 2007; Mahde et al., 2023).

Heimke et al. (2022) confirmed results similar to our research results, and the reason was the anti-inflammatory effects of empagliflozin, measured at the protein level. When primary microglia were stimulated with lipopolysaccharide (LPS) for 24 hours, there was a significant increase in the protein expression of IL-1 and TNF. However, co-treatment with empagliflozin significantly reduced the LPS-induced protein release of IL-1 and TNF (Al-Garawi et al., 2022; Ali et al., 2023).

The nanochitosan group with basil seed extract gave better results due to the nano property. This nanoencapsulation technique improved the physical stability of the linolenic fatty acid of *O. basilicum*. Therefore, it will provide better results than the extract and chitosan alone.

Conclusion

Our findings suggest the capacity of this combination as a therapeutic agent for health improvement and disease prevention, particularly diabetes in male rats. Conversely, the individual use of nanochitosan or basil seed extract alone was less effective in lowering blood sugar, interleukin, and tumor necrotic factor levels while raising insulin levels, highlighting the enhanced delivery capabilities of nanochitosan for herbal substances. The alcoholic extract of *O. basilicum* seeds, when loaded on chitosan, resulted in a decrease in inflammatory markers such as IL-1 β and TNF- α , and an increase in insulin concentration, when elevated, reduces the inflammatory response.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the University of Kufa, Kufa, Iraq (Code: UK.VET.2023.27153).

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interpretation of the results and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

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