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Research Paper

Effects of *Heracleum persicum* and *Nigella sativa L.* alcoholic extracts on insulin amyloid formation

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Introduction

Discovering and describing the critical role of proteins in contrast to disease and defects caused by their inactivity is a major interest for scientific studies. Amyloid aggregation and the diseases caused by them, known as neurodegenerative diseases, reveal a new aspect of protein roles in pathogenicity. Due to the lack of clear treatment for these age-related diseases, prevention of amyloid formation would be the most practical way to deal with them. Proteins are responsible for different cells' activity and their function is related to their structure. When something goes wrong during protein folding. Misfolding proteins can be the main triggers for amyloid formation and neurodegenerative diseases. Insulin is a small protein containing 2 disulfide bonds that *have* been used as an amyloid fibril model in many studies. Insulin's great tendency to form amyloid structures in vitro conditions has made this protein a favorable model for amyloid fibril formation studies. In this research, we analyzed the effects of *Heracleum persicum* and *Nigella sativa L.* alcoholic extracts on insulin amyloid formation.

Methods and Materials

Human insulin protein was purchased from Sigma-Aldrich. All compounds used in this research (NaOH, HCl, Na_2HPO_4 were also obtained from Sigma-Aldrich. Phosphate buffer (50 mM) was used as the main solvent. *Heracleum persicum* fruit and the seeds of *Nigella sativa L.* in powder form were obtained from a local grocery in Tajrish-Tehran. 490 ml of ethanol were added to 100 grams of each powder and then were shaken and incubated at 37 ^oC for 72 hours. After 72 hours samples were filtered by using Whatman papers. Remained alcohol was removed from samples by Rotary evaporator at 50 ^oC. Final extracts were diluted to 25, 50, 100, and 200 µg/ml with phosphate buffer. Insulin protein concentrations were calculated with UV-absorbance and Beer-Lambert law (wavelength= 280 nm and ε = 1,0675). Insulin protein in 2 mg/ml concentration was prepared in phosphate buffer. Insulin pH was adjusted to 5.4 by using HCl and then the sample was incubated at 60 ^oC for 5 hours to form amyloid fibrils. For determining fibril formation or inhibition we applied the fluorescence-Thioflavin T method. Thioflavin T in 5 µM concentration was prepared and then 40 µl of this compound was added to 2ml of insulin solvate. Fluorescence intensity was measured with a fluorescence spectrophotometer (Shimadzu RFg000). *The excitation* wavelength was 440 nm and EX and EM slits were 2.5 and 5 nm. Increasing

fluorescence intensity was taken as a sign of amyloid formation. We also used scanning electron microscopy (SEM) to visualize amyloid fibrils formation.

Results & Discussion

Our results reveal that insulin protein will form amyloid structures at pH= 5.4 and 5 hours of incubation at 60oC. These results were confirmed with SEM and fluorescence-Thioflavin T studies.

Heracleum persicum and *Nigella sativa L*. extractions were added to Insulin protein before putting it in amyloid formation conditions. We used 25, 50, 100, and 200 µg/ml of these extracts and then evaluate amyloid fibril formation with fluorescence-Thioflavin T and SEM microscopy. Fluorescence study results show that our extracts have decreased insulin amyloid formation. We obtained the best inhibitory results while using the highest extract concentration (200 µg/ml). Heracleum persicum shows better inhibition properties than Nigella sativa L extract.

To confirm these inhibitory results, we have used SEM microscopy to visualize insulin amyloid fibril inhibition while adding different concentrations of Heracleum persicum and Nigella sativa L extracts. Microscopic studies show no significant amyloid formation in a higher concentration of our extracts but some amyloid fibril structures were seen when we used the lowest concentration of Nigella sativa L extract, which confirms our fluorescence-ThT studies.

Amyloid fibrils have played a major role in many different neurodegenerative diseases. Amyloid structures of insulin also have been reported in diabetic patients at their insulin injection spots. These concerns persuade us to study insulin amyloid formation and to investigate new possible inhibitors for amyloid fibrils. Fortunately, both plants used in this study have shown great amyloid inhibitory properties. We hopefully recommend that these extracts are valuable candidates for further amyloid and neurodegenerative disease inhibition studies.

Conclusion

Amyloid is a widespread and equally unknown complication, the dependence of many diseases, including diseases that destroy the nervous system. Until now, many compounds have been investigated in terms of their effective properties in preventing amyloid aggregation.

Our results reveal that there is great potential in plant extracts to investigate new amyloid inhibitory drugs. Both of our candidate plant extracts have shown great amyloid inhibitory properties while *Heracleum persicum* extract has shown even better inhibitory potential than *Nigella sativa L*. extract. We suggest that further studies of these extracts may lead to the discovery of new compounds with amyloid inhibitory properties.

Keywords: *Alzheimer's disease, Amyloid fibrils, Heracleum persicum, Nigella sativa L.*

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Declaration of conflict of interest: *The authors declare that they have no conflicts of interest.*