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Research Article

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DOI: https://doi.org/10.21203/rs.3.rs-579314/v1

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Decelerate of amyloid fibrillation by the alkaloids extracted from *Stephania venosa*

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ABSTRACT

Naturally occurring phytochemical compounds have received considerable attention as alternative candidates for anti-amyloidogenic agents. This study, utilizing human insulin and amyloid beta peptide as an *in vitro* model, determined the anti-amyloid effects of alkaloids extracted derived from *Stephania venosa*. Alkaloids extracts including crebanine, O-methylbulbocapnine, tetrahydropalmatine and N-methyltetrahydropalmatine were used. Inhibition of amyloid protein aggregation were studied by fluorescence spectroscopy. Most alkaloids, except N-methyltetrahydropalmatine, exhibited the inhibitory properties against amyloid fibrillation either insulin or amyloid-beta peptide. Among alkaloids group, crebanine and tetrahydropalmatine showed the potent properties of anti-amyloidogenesis. These results suggest that alkaloids could be used as a natural compound for the development of drugs against amyloid protein aggregation for treatment of amyloid-related diseases.

Keywords: amyloid fibrillation, alkaloid, tyrosine, Thioflavin-T

Introduction

Suppression of amyloid protein aggregation is considered as a promising therapeutic approach to prevent or treat amyloidosis-related disorders. One of the strategies aimed to find the effective compound against amyloidogenic activity is to inhibit the toxic amyloid formation and stabilize its native monomeric form or destabilizing the fibrillated misfold form¹. The protein aggregation phenomena can be used as a model for studying proteins’ properties. The ability of various peptides and proteins undergo self-aggregation that leads to the formation of amyloid fibrils. Human insulin is one protein that was chosen and widely used as a model protein for the study of amyloid formation *in vitro*. Recently, much attention has been paid to find out an inhibitor of insulin amyloid fibrils.
In recent years, natural products are identified and characterized as an impact class of amyloid inhibitor. Several natural polyphenolic compounds have been well-studied as amyloid inhibitors such as epigallocatechin gallate (EGCG), curcumin and resveratrol. Quinones show different inhibitory effects on insulin oligomerization, especially for 1,4-benzoquinone and 1,4-naphthoquinone. Quercetin dose-dependently inhibited amyloid formation of insulin via destabilizing the preformed insulin fibrils and transforming the fibrils into amorphous aggregates.

Alkaloids, a class of nitrogen-containing compounds, are found primarily in plants, especially in flowering plants. Alkaloids have a broad spectrum of pharmacological effects including analgesic, antiasthmatic, antiarrhythmic, anticancer etc. Galantamine, the isoquinoline alkaloid family, inhibits Aβ-aggregation and cytotoxicity. However, the common side effects of galantamine can be nausea, vomiting, diarrhea, and anorexia. The in vitro and in vivo study showed that amyloid beta (Aβ) and Aβ-induced neurotoxicity were reduced by the effect of caffeine. In addition, caffeine also reduces levels of Aβ in neuroblastoma-2a cells stably expressing human Swedish mutant APP and protects cerebellar granule neurons and basal forebrain neurons from neurotoxicity caused by Aβ. Furthermore, it should find out an additional alkaloid, either novel compounds or an old one, that could be used for inhibiting Aβ-aggregation.

*Stephania venosa* (Blume) Spreng., belongs to Menispermaceae family. In South East Asian countries, this plant is traditionally used as tonic drug and medication for various diseases. It was found that alkaloids are the main phytochemical compound of this genus. Their biological activities have been reported including anti-cancer activity, chemosensitizer and acetylcholinesterase inhibition. Crebanine, a major component of *S. venosa*, exhibits anti-proliferative and anti-invasive effects on human cancer cells via the cell cycle arrest and apoptosis induction in K562, K562/adr, GLC4 and reduce the expression of MMP-2, MMP-9, uPA and MT1-MMP. In additions, there are several natural alkaloids, including O-methylbulbocapnine, tetrahydropalmatine, and N-methyl tetrahydropalmatine have been found in the tubes of *S. vernosa*. Furthermore, tetrahydropalmatine inhibits LPS-induced IL-8 secretion via blocking MAPKs signaling pathway. However, there is no report about the anti-amyloid effects of these alkaloids.

In the present study, the effect of alkaloid molecules on protein aggregation were investigated using the amyloid-forming model. Human insulin and amyloids beta peptide fibril formation were generated by incubating at high temperature and acid environment (pH 2.5 and 80°C) and the effect of alkaloids on this fibrillation was measured by intrinsic Tyrosine (Tyr) fluorescence assay and Thioflavin T assay. Therefore, the prevention capacity of alkaloids for amyloid fibrils conversion can be indicated as a therapeutic potential on protein aggregation diseases.

**Results**

**Alkaloids extracts inhibited the kinetic of insulin aggregation**

To determine whether alkaloids extracts inhibited insulin fibrillation, tyrosine emission spectra of insulin (0.02 mg.mL⁻¹) in the presence of alkaloids (0.002 mg.mL⁻¹) was observed over a period of 24 h. In the presence of insulin fibrillation, the fluorescence intensity of Tyr was decreasing. This becomes apparent on plotting the fluorescence intensity at 305 nm as a function of time with the half time value of insulin fibrillation (t₁/₂) equal to 0.55 ± 0.11 hour. However, addition of crebanine (A1), O-methylbulbocapnine (A2), tetrahydropalmatine (A3) and N-methyltetrahydropalmatine (A4) to the insulin did not decreased in intensity of the emission at 306 nm over time of incubation. Therefore, the half-time (t₁/₂) of A1, A2, and A4 significantly increased except A3.

**Alkaloids extracts inhibited insulin fibril formation**

To confirm the presence of insulin fibril formation, Thioflavin T (ThT) fluorescence assay was performed. It was found that ThT binds specifically to the cross-β sheet structure of amyloid fibers and give more intense once bond. In this experiment, after incubation for 24 hours, 20 mM ThT was added and measured the fluorescence intensity at 488 nm after excited with 420 nm. The fluorescence intensity of ThT of insulin incubated with A1, A2 and A3 after incubation was lower than that of insulin control with the relative ThT fluorescence equal to 0.44 ± 0.06, 0.59 ± 0.05 and 0.57 ± 0.07, respectively (Table 1). Interestingly, ThT
fluorescence intensity of A4 incubated with insulin did not change when compared with insulin control. Therefore, all alkaloid extracts, except A4, inhibited insulin fibril formation.

Table 1 Effect of alkaloids on insulin fibrillation detected by Tyr fluorescence and Thioflavin T, $^{*}P<0.05$ versus insulin control

| Alkaloids    | Tyrosine fluorescence | Relative ThT fluorescence |
|--------------|-----------------------|---------------------------|
|              | $t_{0.5}^{(s)}$, h    | $\Delta F_{\text{tyr}}$  |
| Insulin      | 0.55 ± 0.11           | 88.0 ± 4.7                | 1.00 |
| Insulin +A1  | 0.81* ± 0.01          | 25.2* ± 4.6               | 0.44* ± 0.06 |
| Insulin +A2  | 0.89* ± 0.09          | 16.9* ± 4.6               | 0.59* ± 0.05 |
| Insulin +A3  | 0.69 ± 0.07           | 61.5* ± 10.6              | 0.57* ± 0.07 |
| Insulin +A4  | 0.79* ± 0.09          | 33.5* ± 4.5               | 1.29 ± 0.27 |

Alkaloids extracts inhibited the insulin fibril formation in dose-dependent manner

The previous data demonstrated that most alkaloids, except A4, had a potential as an inhibitor for insulin fibrillation. We performed further experiment to determine whether alkaloid extracts affect the insulin fibrils formation in dose dependent manner, different concentrations of A1-A4 (0.002-0.01 mg.mL$^{-1}$) were added into insulin (0.02 mg.mL$^{-1}$) prior to warming them to 80 °C for 24 h. After incubation, 20 µM ThT was added and the fluorescence intensity at 488 nm (excitation wavelength at 420 nm) were obtained. Relative ThT fluorescence values were calculated which derived from the ratio of ThT fluorescence intensity of insulin in the presence of alkaloids and the ThT fluorescence intensity of fibrils insulin control. Increased concentration of alkaloid extracts, it was found that A1, A2 and A3 was potently inhibited insulin fibril formation in a dose-dependent manner, while A4 did not. Among alkaloids molecules (at 0.002 mg. mL$^{-1}$), A1 might be the most inhibitor of insulin fibrillation. (Figure 1)
Figure 1 Effect of various concentration of alkaloid extracts, crebanine (a), O-methylbulbocapnine (b), tetrahydropalmatine (c) and N-methyltetrahydropalmatine (d), on insulin fibrillation detected by thioflavin T. The data were presented as mean ± S.D. (n = 3). *P<0.05 versus insulin control.

Alkaloids extracts inhibited the Aβ fibrillation

To characterize the process of Aβ fibrillation, the kinetic of fibrillization process of Aβ peptide with different mixing ratios of Aβ40 to Aβ42 were performed. Our model based on the finding that there are two main Aβ peptides of different length which involved in Alzheimer’s disease, Aβ40 and Aβ42 residues. It was found that mixing of Aβ40 and Aβ42 is enhanced toxicity in the early onset of some familial Alzheimer diseases 20. Otherwise, our previous study found that the ratio of Aβ40: Aβ42 (1:4) increased the toxicity in neuroblastoma cell line, SK-N-SH, than treated with Aβ40 or Aβ42 alone (data not shown). To mimic the pathology of AD, three Aβ peptides were prepared in 1 µM Aβ40, 1 µM Aβ42, and a combination of Aβ40:Aβ42 (0.2 µM:0.8 µM). The fibrillization of Aβ was observed by an increase in ThT fluorescence due to the binding of the dye to the fibrils. The representative fibrillization curves were shown in Figure 2. Our results showed that Aβ40, Aβ42 and Aβ40:Aβ42 demonstrated similar fibrillation kinetics which exhibit a sigmoidal appearance. It seems to be that the fibril growth rate which represented by the half time value of amyloid beta (t0.5) for Aβ42 was shorter than that of Aβ40, indicating Aβ42 exhibited a fast fibrillation rate than Aβ40. Mixing of Aβ40 to Aβ42 seems to decrease the fibril growth rate of Aβ42 comparing with Aβ42 alone (Figure 2d).
Figure 2 Kinetic of amyloid beta fibrillation detected by Thioflavin T. ThT fluorescence emission at 488 nm was monitored upon excitation at 420 nm. The Aβ concentration was performed in 1 µM Aβ40 (a), 1 µM Aβ42 (b), and a combination of Aβ40:Aβ42 (0.2 µM:0.8 µM) (c). All samples were added in 250 µL of PBS buffer pH 7.4 with 0.05% SDS containing 10 µM of ThT, and then incubated at 40°C.

In the presence of alkaloid extracts (0.002 mg. mL⁻¹), the results showed that different alkaloid extracts showed different effect on the kinetic of amyloid beta fibrillations as indicated by half time ($t_{0.5}^{A\beta}$) and the relative ThT fluorescence value as shown in Table 2. Increased half time and decreased relative ThT fluorescence value represent the decreased of amyloid fibrillation formation. The half-time of amyloid formation for Aβ40 was increased from 6.8 h to 8.8 h and 7.7 h in the presence of A1 and A3 respectively. Accordingly, with half-time value, the relative ThT fluorescence value were decreased in the presence of A1 and A3 compared with Aβ control. Therefore, A1 and A3 inhibited the amyloid fibrillation for Aβ40. It was also found that amyloid formation for Aβ42 was inhibited by A2. However, it seems to be that A2 presumably inhibited amyloid formation for Aβ40 and A1 presumably inhibited amyloid formation for Aβ42 as indicated by the decreased of relative ThT fluorescence value comparing with its control.
Table 2 Effect of alkaloids (0.002 mg.mL⁻¹) on Aβ fibrillation detected by Thioflavin T assay. *P<0.05 versus insulin control

| Phytochemicals | Thioflavin T assay |        |        |        |
|----------------|-------------------|--------|--------|--------|
|                | Half time (t_{0.5}^{Aβ}) h | Aβ40   | Aβ42   | Aβ40: Aβ42 |
| Aβ Control     | 6.8 ± 2.7         | 3.9 ± 0.7 | 5.5 ± 0.9 | 1.00   |
| Aβ +A1         | 8.8 ± 1.9         | 3.9 ± 1.2 | 5.0 ± 1.8 | 0.20* ± 0.03 |
| Aβ +A2         | 6.4 ± 2.7         | 8.4 ± 8.0 | 6.3 ± 1.2 | 0.55* ± 0.12 |
| Aβ +A3         | 7.7 ± 3.3         | 5.2 ± 0.9 | 9.3 ± 2.7 | 0.67* ± 0.07 |
| Aβ +A4         | 9.0 ± 0.6         | 4.4 ± 3.4 | 4.3 ± 1.2 | 1.25 ± 0.05 |

Discussion

The incidence of amyloid-related diseases has been growing continuously. Finding an effective treatment became more important. Amyloid fibrillated protein has been being the focus of research for many years.21 An ever-growing incidence of amyloid-related diseases has led researchers and clinicians to discover the cure. Hence, the propose of drugs that prevented amyloid accumulation may be a potential treatment. Recent researches have focused on natural products to avoid the side effect of the clinical used.22 Natural products such as flavonoids, alkaloids and curcuminoids have been extensively researched regarding to reduce the amyloid associated toxicity of Aβ.23,24 In this study, we have proposed alkaloids as an in-situ inhibitor for amyloid protein fibrillation. Four alkaloids derived from Stephania venosa including crebanine, O-methylbulbocapnine, tetrahydropalmatine and N-methyltetrahydropalmatine were used as interested molecules.

We first studied the interaction of alkaloids with human insulin. The reasons why human insulin was chosen as the model protein in this study are as follows; 1) insulin and Aβ protein share a common characteristic. 2) Under appropriate conditions, they both aggregate into amyloid fibrils. 3) Although the proteins do not share sequence homology, they exhibit similar insoluble filaments and fibrillation responses.25,26 According to the process of insulin aggregation, it proceeds through the dissociation of oligomeric states into monomers, results in conformational changes. This change leads to a stable state by forming fibrous amyloid aggregates rich in β-sheets.27 In the present work, the aggregation kinetics of human insulin was studied at low pH and high temperatures. Decrease in Tyr fluorescence intensity, an intrinsic fluorophore, was monitored during insulin aggregation that accompany with insulin fibrillation using Thioflavin T. Our results clearly found that most alkaloids inhibited insulin aggregation and fibril formation as dose dependent manner. O-methylbulbocapnine is isomeric with crebanine with different position of the two methoxyl.13 Both alkaloid molecules exhibited the similar properties of anti-insulin fibrillation. Therefore, different position of the two methoxyl did not affect to the anti-amyloidogenic properties. Interestingly, it was found that the different properties of anti-insulin fibrillation between N-methyltetrahydropalmatine and tetrahydropalmatine. N-methyltetrahydropalmatine is an analogue of tetrahydropalmatine. We found that the methyl group on the nitrogen atom of N-methyltetrahydropalmatine decrease the capacity of insulin fibril formations. Therefore, the nitrogen atom on tetrahydropalmatine seems presumably to play a role as active site for an inhibitor of amyloid fibril formation.

We successfully demonstrated the ability of alkaloids to inhibit the kinetics of insulin aggregation. We postulated that a similar strategy could be used to study on amyloid beta peptide. According to the evidence of the major form of the Aβ peptide that found in amyloid plaque that showed Aβ40 and Aβ42 form mixed aggregates.28 It attempted us to investigate the influence of each Aβ peptide on their aggregation kinetics.
behavior. Kinetic analysis found that Aβ_{42} exhibited a fast fibrillation rate than Aβ_{40}. However, mixing of Aβ_{40} to Aβ_{42} seems to slow down the fibril growth rate of Aβ_{42} when comparing with Aβ_{42} alone. The study from Pauwels et al., used the NMR experiments for visualizing the spontaneous aggregation of mixing Aβ_{40} to Aβ_{42}. It was showed that Aβ_{40} slows down the aggregation kinetics of Aβ_{42}.

In conclusion, amyloid fibrillation could be monitored by using intrinsic Tyrosine fluorescence accompany with Thioflavin T assay. Alkaloids have shown some promise against amyloid fibrils both in insulin and amyloid beta peptide. Most alkaloids group, except N-methyltetrahydropalmatine, exhibited potent properties of anti-amyloidogenesis. These results suggest alkaloid can be used as the natural compound for the development of drugs against amyloid protein aggregation for treatment of Alzheimer’s disease.

**Methods**

**Chemical reagents**

Recombinant human insulin was purchased from Gibco, Life technology. Before the experiments, insulin solution was diluted in 0.025 M HCl, 0.1 M NaCl pH 1.6. Amyloid beta peptides, i.e. Aβ_{40} and Aβ_{42}, were purchased from EZBiolab Laboratories and were initially solubilized, in 1,1,1,3,3,3-hexafluoro-2-propanol or hexafluoroisopropanol (HFIP) (Fluka). Stock solution 1 mM in HFIP was aliquoted in 20 μL in each microtube and dried under N_{2} gas atmosphere to undergo dried films, then stored at −20 °C. Before performed the experiments, aliquots were resuspended at a final concentration of 5 mM in DMSO (Sigma), sonicated using a bath sonicator for 10 min, and diluted to 100 μM with a phosphate buffer solution (PBS) plus 0.05 % sodium dodecyl sulfate (SDS) (Sigma). Thioflavin T (ThT) (Sigma) was dissolved in PBS pH 7.4 and filtered through a 0.2 μm syringe filter. The concentration of ThT was determined using UV absorbance at 412 nm and calculated by using the extinction coefficient of 36,000 M⁻¹cm⁻¹. Four alkaloids including crebanine (A1), O-methylbulbocapnine (A2), tetrahydropalmatine (A3) and N-methyltetrahydropalmatine (A4) were kindly provided by Associated Professor Dr. Wilart Pompimon, Department of Chemistry, Faculty of Science, Lampang Rajabhat University, Thailand. Those four alkaloids were extracted from S. venosa tuber that was collected and identified by Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok, Thailand with a specimen number of BKF 140583. The crebanine and tetrahydropalmatine were isolated from ethyl acetate extract, while the O-methylbulbocapnine and N-methyltetrahydropalmatine isolated from acetone extract. All alkaloids’ molecules were dissolved in DMSO. (Figure 3) Other chemical reagents include MEM, HAM/F12 medium with L-glutamine (Caisson, USA), DMEM with high-glucose and L-glutamine (Caisson, USA), Penicillin-Streptomycin (Caisson, USA), Fetal bovine serum (Gibco®, Invitrogen, USA), Dimethyl sulfoxide (DMSO).
Kinetics of insulin fibrillation by intrinsic Tyrosine fluorescence

The fluorescence intensity of tyrosine (Tyr) was used to investigate the insulin fibrillation. The insulin fibrillation was performed by using the thermal-induced fibrillation method. The experiment was assigned by incubating 2 mL of insulin (0.02 mg.mL⁻¹) or insulin with alkaloids (0.002 mg.mL⁻¹) at 80 °C over a period of 24 h. The emission spectra of Tyr were recorded from 280 to 500 nm in a 1cm quartz cell by exciting at 276 nm. The fluorescence intensity of Tyr at 306 nm was plotted against time of incubation. Efficiency of alkaloids to inhibit the insulin fibrillation was assessed by two terms; 1) the half time value of insulin fibrillation (tₐₐ₅₀ ins) and 2) the altered fluorescence intensity of tyrosine (ΔFₜₜᵣ). The half time value of insulin fibrillation (tₐₐ₅₀ ins) defined as the time where the signal has reached 50% of the amplitude of the transition ($A/2=(F_n-F_i)/2$) that as shown in Figure 4a. Where $F_i$ and $F_n$ are the fluorescence intensities at the initial reaction and at steady state, respectively, and $A$ is the amplitude of reaction. The altered fluorescence intensity (ΔFₜₜᵣ) signified the amount of insulin fibril formation and defined the percentage of the diminution of fluorescence intensity ($A/F_i*100$). (Figure 4a)

Effect of alkaloids on insulin fibrillation

Various concentration of alkaloids (0.002-0.01 mg.mL⁻¹) were added into insulin solutions (0.02 mg.mL⁻¹) prior to warm at 80 °C for 24 h. After incubation, 20 µM ThT was added and the fluorescence emission spectra (excitation wavelength at 420 nm) were obtained. Relative ThT fluorescence value were calculated from the ratio of ThT fluorescence intensity of insulin in the presence of alkaloids and insulin control.

Kinetic analysis of amyloid fibrillation

The Aβ fibrillation was performed in 1 µM Aβ₄₀, 1 µM Aβ₄₂, and a combination of Aβ₄₀:Aβ₄₂ (0.2 µM:0.8 µM). All samples were added in 250 µL of PBS buffer pH 7.4 with 0.05% SDS containing 10 µM of ThT, and then incubated at 40°C. The fluorescence intensity of ThT was taken using a spectrofluorometer (Perkin Elmer LS55) with the emission wavelength at 488 nm excitation wavelength at 420 nm.

Effect of alkaloids on Aβ fibrillation

The experiments were performed by co-incubating of Aβ₄₀ (1µM), Aβ₄₂ (1µM) or Aβ₄₀:Aβ₄₂ (0.2:0.8 µM) with alkaloids (0.002 mg.mL⁻¹) at 40 °C and used 10 µM of ThT for fibrillation analysis. The fluorescence intensity of ThT was measured at 488 nm when excitation at 420 nm using a spectrofluorometer. The kinetics of Aβ fibrillationization could be described as sigmoid curves and the aggregation parameters were determined by fitting the plot of fluorescence intensity versus time as indicated in Figure 4b. The fibrillation rate presented in the haft time value of amyloid beta ($t_{0.5}^{AB}$) was used for data analysis. Efficiency of alkaloids to inhibit the Aβ fibrillation was assessed by two terms; 1) the half time value ($t_{0.5}^{AB}$) defined as the time where the signal has reached 50% of the amplitude ($A$) of the transition ($A/2=(F_n-F_i)/2$). Where $F_i$ and $F_n$ are the fluorescence intensities at the initial reaction and at steady state, respectively, and $A$ is the amplitude of reaction. (Figure 4b)

Statistical analysis

All data are expressed as mean ± standard deviation (SD). Statistical significance was determined using Student's t-test between the groups treated and the control. A probability (p) value less than 0.05 was considered statistically significant.
Figure 4 The kinetic of insulin fibrillation monitoring by the fluorescence intensity of tyrosine. (a) and the kinetic of amyloid beta fibrillation monitoring by the fluorescence intensity of Thioflavin T. (b)

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Acknowledgements

The authors would like to thank Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, 50200, Thailand. (This work was a part of K.D.’s Ph.D. dissertation.)

Author contributions statement

C.U. and K.D. conducted the experiments, W.P. prepared 4 alkaloids, C.U., K.D., M.S., and W.P. analyzed the results. All authors reviewed the manuscript.

Table 1 Effect of alkaloids on insulin fibrillation detected by Tyr fluorescence and Thioflavin T, *P<0.05 versus insulin control

Table 2 Effect of alkaloids (0.002 mg.mL\(^{-1}\)) on A\(\beta\) fibrillation detected by Thioflavin T assay, *P<0.05 versus insulin control

Figure 1 Effect of various concentration of alkaloid extracts, crebanine (a), O-methylbulbucapnine (b), tetrahydropalmatine (c) and N-methyltetrahydropalmatine (d), on insulin fibrillation detected by thioflavin T. The data were presented as mean \(\pm\) S.D. (n = 3). *P<0.05 versus insulin control

Figure 2 Kinetic of amyloid beta fibrillation detected by Thioflavin T. ThT fluorescence emission at 488 nm was monitored upon excitation at 420 nm. The A\(\beta\) concentration was performed in 1 \(\mu\)M A\(\beta_{40}\) (a), 1 \(\mu\)M A\(\beta_{42}\)(b), and a combination of A\(\beta_{40}\):A\(\beta_{42}\) (0.2 \(\mu\)M:0.8 \(\mu\)M)(c). All samples were added in 250 \(\mu\)L of PBS buffer pH 7.4 with 0.05% SDS containing 10 \(\mu\)M of ThT, and then incubated at 40°C.

Figure 3 Chemical structure of four alkaloids derived from Stephania venosa (Blume) Spreng

Figure 4 The kinetic of insulin fibrillation monitoring by the fluorescence intensity of tyrosine. (a) and the kinetic of amyloid beta fibrillation monitoring by the fluorescence intensity of Thioflavin T. (b)
Effect of various concentration of alkaloid extracts, crebanine (a), O-methylbulbocapnine (b), tetrahydropalmatine (c) and N-methyltetrahydropalmatine (d), on insulin fibrillation detected by thioflavin T. The data were presented as mean ± S.D. (n = 3). *P<0.05 versus insulin control
Figure 2

Kinetic of amyloid beta fibrillation detected by Thioflavin T. ThT fluorescence emission at 488 nm was monitored upon excitation at 420 nm. The Aβ concentration was performed in 1 μM Aβ40 (a), 1 μM Aβ42(b), and a combination of Aβ40:Aβ42 (0.2 μM:0.8 μM)(c). All samples were added in 250 μL of PBS buffer pH 7.4 with 0.05% SDS containing 10 μM of ThT, and then incubated at 40°C.
Figure 3

Chemical structure of four alkaloids derived from Stephania venosa (Blume) Spreng
Figure 4

The kinetic of insulin fibrillation monitoring by the fluorescence intensity of tyrosine. (a) and the kinetic of amyloid beta fibrillation monitoring by the fluorescence intensity of Thioflavin T. (b)