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Krai Daowtak

Naresuan University

Wilart Pompimon

Lampang Rajabhat University

Milena Salerno

Université Sorbonne Paris Nord, équipe NBD, UMR 7244

Chatchanok Udomtanakunchai (✉ chatchanok.u@cmu.ac.th)

Chiang Mai University

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

Krai Daowtak¹, Wilart Pompimon², Milena Salerno³, and Chatchanok Udomtanakunchai^{4,*}

¹Department of Medical Technology, Faculty of Allied Health Sciences, Naresuan University, Phitsanulok, 65000, Thailand

²Laboratory of Natural Products, Department of Chemistry, Faculty of Science, Lampang Rajabhat University, Lampang, 52100, Thailand

³Université Sorbonne Paris Nord, équipe NBD, UMR 7244, Bobigny, F-93022, France

⁴Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, 50200, Thailand

*Corresponding author e-mail: chatchanok.u@cmu.ac.th

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 form ¹. 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 ¹. 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 ². Quercetin dose-dependently inhibited amyloid formation of insulin *via* destabilizing the preformed insulin fibrils and transforming the fibrils into amorphous aggregates ³.

Alkaloids, a class of nitrogen-containing compounds, are found primarily in plants, especially in flowering plants ⁴. Alkaloids have a broad spectrum of pharmacological effects including analgesic, antiasthmatic, antiarrhythmic, anticancer etc. ^{4,5}. Galantamine, the isoquinoline alkaloid family, inhibits A β -aggregation and cytotoxicity ^{4,6}. However, the common side effects of galantamine can be nausea, vomiting, diarrhea, and anorexia. ⁷. The *in vitro* and *in vivo* study showed that amyloid beta (A β) and A β -induced neurotoxicity were reduced by the effect of caffeine ⁸. In addition, caffeine also reduces levels of A β in neuroblastoma-2a cells stably expressing human Swedish mutant APP and protects cerebellar granule neurons and basal forebrain neurons from neurotoxicity caused by A β ^{8,9}. Furthermore, it should find out an additional alkaloid, either novel compounds or an old one, that could be used for inhibiting A β -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 ¹⁰. It was found that alkaloids are the main phytochemical compound of this genus ¹¹. Their biological activities have been reported including anti-cancer activity ^{12,13}, chemosensitizer ¹⁴ and acetylcholinesterase inhibition ¹⁵. 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 ^{13,16-18}. In additions, there are several natural alkaloids, including *O*-methylbulbocapnine, tetrahydropalmatine, and *N*-methyl tetrahydropalmatine have been found in the tubes of *S. vernosa* ^{12,13}. Furthermore, tetrahydropalmatine inhibits LPS-induced IL-8 secretion *via* blocking MAPKs signaling pathway ¹⁹. 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 ($t_{0.5}^{ins}$) equal to 0.55 $\pm$ 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 ($t_{0.5}^{ins}$) 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 bound. 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 $\pm$ 0.06, 0.59 $\pm$ 0.05 and 0.57 $\pm$ 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
	Half time ($t_{0.5}^{ins}$), h	ΔF_{tyr}	
Insulin	0.55 ± 0.11	88.0 ± 4.7	1.00
Insulin +A1	$0.81^* \pm 0.01$	$25.2^* \pm 4.6$	$0.44^* \pm 0.06$
Insulin +A2	$0.89^* \pm 0.09$	$16.9^* \pm 4.6$	$0.59^* \pm 0.05$
Insulin +A3	0.69 ± 0.07	$61.5^* \pm 10.6$	$0.57^* \pm 0.07$
Insulin +A4	$0.79^* \pm 0.09$	$33.5^* \pm 4.5$	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 $\pm$ S.D. ($n = 3$). * $P < 0.05$ versus insulin control

Alkaloids extracts inhibited the A β fibrillation

To characterize the process of A β fibrillation, the kinetic of fibrillization process of A β peptide with different mixing ratios of A β_{40} to A β_{42} were performed. Our model based on the finding that there are two main A β peptides of different length which involved in Alzheimer's disease, A β_{40} and A β_{42} residues. It was found that mixing of A β_{40} and A β_{42} is enhanced toxicity in the early onset of some familial Alzheimer diseases²⁰. Otherwise, our previous study found that the ratio of A β_{40} : A β_{42} (1:4) increased the toxicity in neuroblastoma cell line, SK-N-SH, than treated with A β_{40} or A β_{42} alone (data not shown). To mimic the pathology of AD, three A β peptides were prepared in 1 μ M A β_{40} , 1 μ M A β_{42} , and a combination of A β_{40} :A β_{42} (0.2 μ M:0.8 μ M). The fibrillization of A β 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 A β_{40} , A β_{42} and A β_{40} :A β_{42} demonstrated similar fibrillization 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 ($t_{0.5}^{A\beta}$) for A β_{42} was shorter than that of A β_{40} , indicating A β_{42} exhibited a fast fibrillation rate than A β_{40} . Mixing of A β_{40} to A β_{42} seems to decrease the fibril growth rate of A β_{42} comparing with A β_{42} 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 A β fibrillation detected by Thioflavin T assay, * $P < 0.05$ versus insulin control

Phytochemicals	Thioflavin T assay					
	Half time ($t_{0.5}^{A\beta}$), h			Relative ThT fluorescence		
	A β_{40}	A β_{42}	A β_{40} : A β_{42}	A β_{40}	A β_{42}	A β_{40} : A β_{42}
A β Control	6.8 $\pm$ 2.7	3.9 $\pm$ 0.7	5.5 $\pm$ 0.9	1.00	1.00	1.00
A β +A1	8.8 $\pm$ 1.9	3.9 $\pm$ 1.2	5.0 $\pm$ 1.8	0.20* $\pm$ 0.03	0.70 $\pm$ 0.05	1.02 $\pm$ 0.11
A β +A2	6.4 $\pm$ 2.7	8.4 $\pm$ 8.0	6.3 $\pm$ 1.2	0.55* $\pm$ 0.12	0.93 $\pm$ 0.38	1.03 $\pm$ 0.18
A β +A3	7.7 $\pm$ 3.3	5.2 $\pm$ 0.9	9.3 $\pm$ 2.7	0.67* $\pm$ 0.07	1.01 $\pm$ 0.06	1.54 $\pm$ 0.74
A β +A4	9.0 $\pm$ 0.6	4.4 $\pm$ 3.4	4.3 $\pm$ 1.2	1.25 $\pm$ 0.05	1.35 $\pm$ 0.25	1.58 $\pm$ 0.04

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 years²¹. 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 used²². Natural products such as flavonoids, alkaloids and curcuminoids have been extensively researched regarding to reduce the amyloid associated toxicity of A β ^{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 A β 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 -sheets²⁷. In the present work, the aggregation kinetics of human insulin was studied at low pH and high temperatures. Decreased 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 methoxyl¹³. 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 A β peptide that found in amyloid plaque that showed A β_{40} and A β_{42} form mixed aggregates²⁸. It attempted us to investigate the influence of each A β peptide on their aggregation kinetics

behavior. Kinetic analysis found that A β ₄₂ exhibited a fast fibrillation rate than A β ₄₀. However, mixing of A β ₄₀ to A β ₄₂ seems to slow down the fibril growth rate of A β ₄₂ when comparing with A β ₄₂ alone. The study from Pauwels *et al.*, used the NMR experiments for visualizing the spontaneous aggregation of mixing A β ₄₀ to A β ₄₂. It was showed that A β ₄₀ slows down the aggregation kinetics of A β ₄₂²⁹.

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. A β ₄₀ and A β 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 μ L in each microtube and dried under N₂ 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 μ M 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 μ m 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 extract¹³. 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).

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

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 method³⁰. 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 ($t_{0.5}^{ins}$) and 2) the altered fluorescence intensity of tyrosine (ΔF_{Tyr}). The half time value of insulin fibrillation ($t_{0.5}^{ins}$) defined as the time where the signal has reached 50% of the amplitude of the transition ($A/2=(F_i-F_n)/2$) 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 (ΔF_{Tyr}) 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 μ M 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 A β fibrillation was performed in 1 μ M A β_{40} , 1 μ M A β_{42} , and a combination of A β_{40} :A β_{42} (0.2 μ M:0.8 μ M). All samples were added in 250 μ L of PBS buffer pH 7.4 with 0.05% SDS containing 10 μ M 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 A β fibrillation

The experiments were performed by co-incubating of A β_{40} (1 μ M), A β_{42} (1 μ M) or A β_{40} :A β_{42} (0.2:0.8 μ M) with alkaloids (0.002 mg.mL⁻¹) at 40 °C and used 10 μ M 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 A β fibrillization 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 half time value of amyloid beta ($t_{0.5}^{A\beta}$) was used for data analysis. Efficiency of alkaloids to inhibit the A β fibrillation was assessed by two terms; 1) the half time value ($t_{0.5}^{A\beta}$) 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 $\pm$ 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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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 β fibrillation detected by Thioflavin T assay, *P<0.05 versus insulin control

Figure 1 Effect of various concentration of alkaloid extracts, crebanine (a), *O*-methylbulbocapnine (b), tetrahydropalmatine (c) and *N*-methyln-tetrahydropalmatine (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 β concentration was performed in 1 μ M A β ₄₀ (a), 1 μ M A β ₄₂ (b), and a combination of A β ₄₀:A β ₄₂ (0.2 μ M:0.8 μ M) (c). All samples were added in 250 μ L of PBS buffer pH 7.4 with 0.05% SDS containing 10 μ 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)

Figures

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 $\pm$ 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 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.

O-methylbulbocapnine

Tetrahydropalmatine

Crebanine

N-methyltetrahydropalmatine

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)