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Biological activities of protein extracts from silkworm pupae against non-communicable diseases

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Abstract

Non-communicable diseases or NCDs, such as cancers, diabetes, hypertension and Alzheimer's disease, are public health issues of our country and lead causes of death. However, NCDs can be prevented. Previous studies have shown that silkworm pupae which are a waste product after silk drawing, contain high quality proteins and can be used in medicine. This research is interested in studying biological activities of protein extracts from silkworm pupae against NCDs. Two species of silkworm pupae were used; Thai-multivoltine silkworm pupae, *Bombyx mori* (Nanglai strain) and Wild silkworm pupae, *Samia ricini* (Eri). The following in vitro tests were designed to determine the biological activities of protein extracts from silkworm pupae including anti-inflammation by inhibition of albumin degradation, anti-diabetic properties by α-amylase and α-glucosidase inhibition tests, antioxidant activities by 2,2-diphenyl-1-picryl hydrazyl (DPPH) and ferrous ion chelating, and anti-acetylcholinesterase activity by quantifying the acetylcholinesterase (AChE) inhibitory activity. Assays were evaluated using inhibitory concentration (IC_{50}) value. The results indicated that protein extracts from Nanglai and also Eri showed all inhibitory activities. Nanglai displayed stronger albumin degradation, α-amylase and acetylcholinesterase inhibition, and higher DPPH radical scavenging activity, whereas Eri exhibited better anti-diabetic activity by inhibiting α-glucosidase and iron chelating activity. The results of this study revealed that protein extracts from silkworm pupae had biological activities against most causes of NCDs, offering a possibility of prevention and treatment of NCDs in the future.

Introduction

Non-communicable diseases (NCDs) such as cancer, diabetes, Alzheimer's and chronic respiratory diseases represent the largest cause of mortality in the Thai population over the past 10 years, and cause a huge economic burden costing (World Health Organization). Prevention of premature deaths due to NCDs and reduction of related health care costs will be the main goals of health policy. Therefore, improving the treatment of NCDs and preventing complications will be the major goals of clinical medicine.

Thailand has the resources to manufacture and produce its own silk, known as sericulture. A silkworm pupae is a by-product from silk drawing. In this research, protein extracts from two types of domesticated silkworm pupae that produce commercial silk, Thai native silkworm; Nanglai *(Bombyx mori*) and wild-silkworm; Eri (non-mulberry silkworm, *Samia ricini*) were investigated for biological activities against NCDs including anti-acetylcholinesterase activity, anti-diabetic properties, anti-inflammation, and antioxidant. Recent studies have found that silkworm pupae are a source of high quality proteins[1] and essential nutrient elements for human[2,3]. They recently have been discovered many benefits for

medicine[4], for example they protect against Alzheimer's disease by decreasing memory impairment and neurons density in hippocampus in animal model[5]. The only symptomatic treatment proven effective to date is the use of acetlycholinesterase inhibitors to augment surviving acetylcholinergic activity[6].

Diabetes is one of the most common chronic diseases characterized by hyperglycemia as a result of impaired insulin secretion by pancreatic β cells and by cellular resistance to insulin[7]. Studies have reported that extracts of silkworm pupa reduce blood glucose in diabetic rats, and decrease fat storage, this is considered to be effective in the inhibition of the metabolic syndrome[8]. Recent data have expanded the concept that inflammation is a critical component of cancer development and progression[9]. Chronic inflammation increases the risk for various cancers, indicating that eliminating inflammation can be used as valid strategy for cancer prevention and therapy[10]. Moreover oxidative stress has been implicated in various pathological conditions involving cancer, neurological disorders (Alzheimer's disease and Parkinson's disease), diabetes, and cardiovascular disease[11]. Recent studies have revealed that silkworm pupae oil exerts hypolipidemic and antioxidant effects in high-cholesterol diet-induced rats[12].

However, there is no report on in vitro anti-inflammatory, anti-diabetic antioxidant and anti-acetylcholinesterase property of protein extracts from *Bombyx mori* and *Samia ricini*. Therefore, the purpose of this study was to investigate the biological activities of protein extracts from silkworm pupae against major causes of NCDs.

Methodology

Protein extraction

Two species of silkworm pupae were used in this study; Thaimultivoltine silkworm, *Bombyx mori* (Nanglai strain) and wild silkworm, *Samia ricini* (Eri) [silkworm and wild silkworm samples supported by Assistant Professor Amornrat Promboon, Department of Biochemistry, Faculty of Science, Kasetsart University, Thailand]. Extraction buffer containing 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% TritonX-100, 0.1% SDS, and 4 mM PMSF was used. After centrifugation, supernatants were dialyzed for 3 hours. Crude protein concentrations were measured using Bradford protein assay (bovine serum albumin (BSA) as standard).

Anti- Acetylcholinesterase activity

Acetylcholinesterase inhibitory activity

Acetylcholinesterase (AChE) inhibitory property was measured modifying the previously reported method (Selles *et al.)* [13]. Protein extracts from Nanglai or Eri at various concentrations (20 µl/ml, 50 µl/ml, 100 µl/ml and 200 µl/ml) were added to 100 mmol/l sodium phosphate buffer (pH 8.0) made up to total volume of 150 µl. 10 µl Acetylcholinesterase (AChE) (1 unit/ml/min) was added and incubated for 15 min at 25° C. The reaction was started by adding 20 µl of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) (0.5 mmol/l) and 10 µl of 15 mM acetylthiocholine iodide. The hydrolysis of this substrate was monitored spectrophotometrically at 412 nm. Galantamine (Acetylcholinesterase inhibitor) was used as a reference compound. The assay was performed in triplicate. The percentage inhibition of AChE activity was calculated using the following formula:

$$\% \text{ Enzyme inhibition activity} = [(A_{\text{control (without extract)}} - A_{\text{sample}}) / A_{\text{control (without extract)}}] \times 100$$

Where A is the absorbance reading measured at 412 nm.

The IC_{50} value was defined as the concentration of the compound required to inhibit 50% of the Acetylcholinesterase activity under the assay conditions.

Anti-Diabetic activity

α-Amylase inhibition assay

α-Amylase inhibitory activity was assayed according to the procedure described by Hansawasdi *et al.*[14] with a slight modification. α-Amylase activity was determined using soluble starch (1%) as a substrate in 0.02 mol/l sodium phosphate buffer (pH 6.9). Protein extracts from Nanglai or Eri at various concentrations (20 µl/ml, 50 µl/ml, 100 µl/ml and 200 µl/ml) were mixed with substrate solution made up to total volume of 150 µl. 10 µl of α-amylase solution (1 unit/ml/min) was added. After incubation at 25 °C for 30 min, 30 µl of Dinitrosalicylic acid reagent was added and incubated at 90 °C for 5 min. The absorbance was measured at 540 nm. Acarbose (α-Amylase inhibitor) was used as a positive control. The assay was performed in triplicate. The percentage inhibition was calculated using the following formula:

% Enzyme inhibition activity = $[(A_{control\ (without\ extract)} - A_{sample}) / A_{control\ (without\ extract)}] \times 100$

Where A is the absorbance reading measured at 540 nm.

The IC_{50} value was defined as the concentration of the compound required to inhibit 50% of the α-Amylase activity under the assay conditions.

α-Glucosidase inhibition assay

The inhibition of α-glucosidase assay is a modification of the method previously described by Lebowitz *et al*.[15] Protein extracts from Nanglai or Eri at various concentrations (20 µl/ml, 50 µl/ml, 100 µl/ml and 200 µl/ml) were mixed with 0.1 mol/l potassium phosphate buffer (pH 6.9) made up to total volume of 150 µl. 10 µl of α-glucosidase solution (1 unit/ml/min). The mixer was incubated at 37 °C for 15 min. Then, 10 mL of 3 mM p-nitrophenyl-α-Dglucopyranoside (PNP-G) was added and the mixture was re-incubated at 37 °C for 10 min. The reaction was terminated by the addition of 30 ml of 0.1 M sodium carbonate. The amount of released product (p-nitrophenol) was measured at 405 nm using a UV spectrophotometer to estimate the enzymatic activity. Acarbose (α-Amylase inhibitor) was used as a positive control. The assay was performed in triplicate. The percentage inhibition was calculated using the following formula:

% Enzyme inhibition activity = $[(A_{control\ (without\ extract)} - A_{sample}) / A_{control\ (without\ extract)}] \times 100$

Where A is the absorbance reading measured at 540 nm.

The IC_{50} value was defined as the concentration of the compound required to inhibit 50% of the α-Glucosidase activity under the assay conditions.

Anti-Inflammation

Albumin denaturation inhibitory assay

The assay was carried out by the methods described by Osman *et al.*[16] with some modifications. Protein extracts from Nanglai or Eri at various concentrations (20 µl/ml, 50 µl/ml, 100 µl/ml and 200 µl/ml) were mixed with Tris buffer (pH 6.4) made up to total volume of 150 µl. Then, 50 µl of 1 mg/ml Bovine serum albumin (BSA) was added. Afterward, the mixtures were incubated at 37°C for 20 min and then heated at 70°C for 5 min. After cooling, their absorbances were measured at 660 nm. Ibuprofen (anti-inflammatory drug) was used as a positive control. The assay was performed in triplicate. The percentage inhibition was calculated using the following formula:

% inhibition of denaturation = $[(A_{control\ (without\ extract)} - A_{sample}) / A_{control\ (without\ extract)}] \times 100$

Where A is the absorbance reading measured at 660 nm.

The IC_{50} value was defined as the concentration of the compound required to inhibit 50% of albumin degradation under the assay conditions.

Antioxidant activity

DPPH radical scavenging activity assay

The antioxidant activity of the compounds was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical 2,2-diphenyl-2-picrylhydrazyl(DPPH•), according to the method of Blois[17]. Protein extracts from Nanglai or Eri at various concentrations (20 µl/ml, 50 µl/ml, 100 µl/ml and 200 µl/ml) were added to 50 mM potassium phosphate buffer (pH 7.0) made up to total volume of 150 µl. 50 µl of 0.125 mg/ml 2,2-diphenyl-1-picrythyhydrazyl radical (DPPH) in 70% ethanol was added. The mixed solution was incubated for 30 min at 25°C. The absorbance of the sample was measured at 517 nm. Ascorbic acid was used as a positive control. The assay was performed in triplicate. The percentage oxidation inhibition was calculated using the following formula:

$$\% \text{ Oxidation inhibition activity} = [(A_{\text{control (without extract)}} - A_{\text{sample}}) / A_{\text{control (without extract)}}] \times 100$$

Where A is the absorbance reading measured at 517 nm.

The IC_{50} value was defined as the concentration of the compound required to reduce 50% of the DPPH radial under the assay conditions.

Iron chelating activity assay

The basic principle of this test is based on the capacity to decolorize the iron-ferrozine complex. Ferrozine is a substance which can quickly act on iron to form a coloured complex. Iron chelating activity was assayed according to the procedure described by Chaudhary *et al.*[18] with a slight modification. The 1, l0-Phenanthroline-iron (III) reagent was prepared by mixing 0.198 g of l, l0-phenanthroline monohydrate, 2 ml of 1 M hydrochloric acid and 0.16 g of ferric ammonium sulphate in 100 ml water. Protein extracts from Nanglai or Eri at various concentrations (20 µl/ml, 50 µl/ml, 100 µl/ml and 200 µl/ml) were mixed with deionized water made up to total volume of 150 µl. Then, 20 µl of prepared 1, l0-Phenanthroline-iron (III) reagent and 30 µl methanol were added. After incubation at 50°C for 30 minutes, the absorbance of the complex was measured at 510 nm. Ascorbic acid was used as positive control. The assay was performed in triplicate. The percentage inhibition was calculated using the following formula:

$$\% \text{ Chelating activity} = [(A_{\text{control (without extract)}} - A_{\text{sample}}) / A_{\text{control (without extract)}}] \times 100$$

Where A is the absorbance reading measured at 510 nm.

The EC_{50} (Effective Concentration) value was defined as the concentration of the compound required to chelate 50 % of ferrous ions, under the assay conditions.

Statistical analysis

Data are represented as the mean ± SEM. The differences were analyzed using one-way ANOVA test. A P value less than 0.05 was considered statistically significant.

Results and Discussion

Anti-Acetylcholinesterase activity

Breakdown of acetylcholine in the hippocampus and cortex of the brain is one of the most important remarkable changes observed in Alzheimer's disease. Acetylcholine reduction in the brain can be prevented by the inhibition of acetyl cholinesterase activity, which subsequently increases the concentration of acetylcholine. The inhibitory activity of protein extracts from silkworm pupae, *Bombyx mori* (Nanglai) and *Samia ricini* (Eri), was evaluated by acetylcholine levels using acetylthiocholine iodide (as substrate) and 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB). The enzymatic activity was measured by the yellow color compound. The results of the acetylcholinesterase inhibitory activity of protein extracts from silkworm pupae are shown in Figure 1 and Table 1.

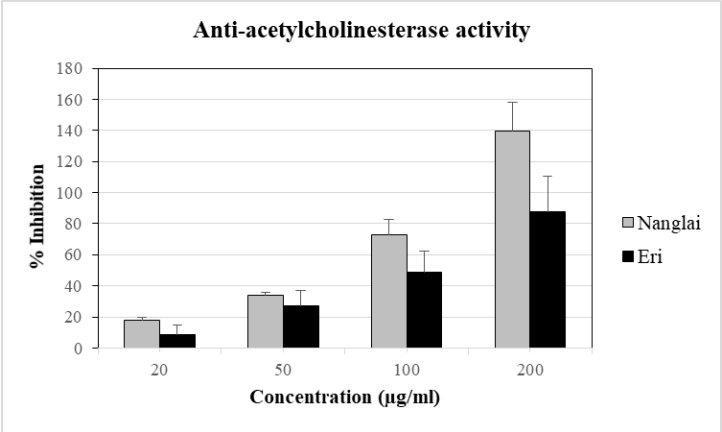

Figure 1. Inhibitory activity against acetylcholinesterase of protein extracts from *Bombyx mori* (Nanglai) and wild silkworm, *Samia ricini* (Eri)

Table 1. Anti- Acetylcholinesterase activity of protein extracts from *Bombyx mori* (Nanglai) and wild silkworm, *Samia ricini* (Eri)

Samples	**Acetylcholinesterase inhibitory activity (IC_{50} µg/ml)**
Bombyx mori (Nanglai)	74.6 ± 2.2^a
Samia ricini (Eri)	113.6 ± 3.1^b
Galantamine (+ control)	53.4 ± 1.2^a

Values are mean of three replicate determinations (n = 3) ± standard deviation. Mean values followed by different superscripts in a column are significantly different (P <0.05; ANOVA, followed by Dunnett's multiple comparison test).

The extracts of both species exhibited anti-acetylcholinesterase property in a dose-dependent manner. Nanglai was found to inhibit acetylcholinesterase activity by 50% at a concentration of 74.6 µg/ml, while Eri inhibited the activity by 50% at a concentration of 113.6 µg/ml. Galantamine, a natural acetylcholinesterase inhibitor used in therapeutics, was used as reference. All the extracts showed lower potent than that of the galantamine (53.4 µg/ml) under the same experimental condition. Our results demonstrated that protein extracts from Nanglai had a slightly potential inhibitory activity against acetylcholinesterase to galantamine. Previous study by Wattanathorn *et al*[5] showed that dietary supplementation with *Bombyx mori* pupae powder could enhance the cholinergic function by decreasing both the cholinergic neurons density and acetylcholinesterase activity in hippocampus. Other studies also indicated that acetylcholinesterase inhibitor could also improve memory impairment in Alzheimer's disease[19]. Therefore, result in increased acetylcholine by these protein extracts could offer a therapeutic effect in patients with Alzheimer's disease.

Anti-diabetic activity

Diabetes mellitus is a complex disease characterized by high concentrations of blood glucose which can cause serious complications in many organs. Therefore, the treatment of diabetes basically focuses on reducing fluctuations in blood glucose. The carbohydrate hydrolyzing enzyme inhibitors, α-amylase and α-glucosidase inhibitors, are currently used for diabetic treatment. Inhibitors of α-amylase and α-glucosidase delay the breakdown of carbohydrate and decrease the postprandial blood glucose levels. The inhibition of these two prominent enzymes has been found as a useful and effective strategy to lower the levels of postprandial hyperglycemia. Acarbose is a commercially available α-amylase and α-glucosidase inhibitor for type II diabetes. However, it is reported to cause various side effects

such as diarrhea and other intestinal disturbances, with corresponding intestinal pain and flatulence. Searching for safe and effective inhibitors from natural sources are of emerging interest.

α-Amylase inhibitory activity

The inhibitory effects of protein extracts from silkworm pupae, *Bombyx mori* (Nanglai) and *Samia ricini* (Eri) on α-amylase are shown in Figure 2. The extracts of both species exhibited anti-α-amylase activity in a dose-dependent manner. The IC_{50} values are revealed in Table 2. The potential inhibition of all extracts against *α*-amylase ranged from 98.1 µg/ml by Nanglai to 125 µg/ml by Eri. However, all the extracts showed lower inhibitory activities compared with that of acarbose (54.8 µg/ml). As expected, acarbose showed the lowest IC_{50}, establishing its relative potency as a glucosidase inhibitor. Nanglai extract also was a stronger inhibitor of α-amylase than Eri extract. These results suggested that α-amylase inhibitory activity of Nanglai had effective delay of starch and oligosaccharide digestion to absorbable monosaccharides that would consequently result in reduced postprandial hyperglycemia.

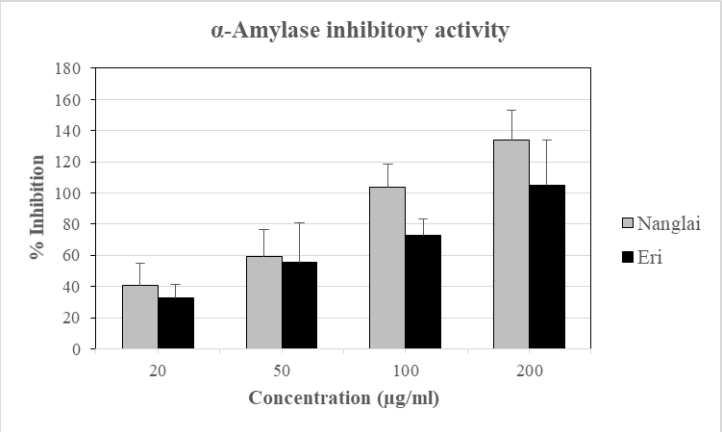

Figure 2. Inhibitory activity against α-Amylase of protein extracts from *Bombyx mori* (Nanglai) and wild silkworm, *Samia ricini* (Eri)

Table 2. α-Amylase inhibitory activity of protein extracts from *Bombyx mori* (Nanglai) and wild silkworm, *Samia ricini* (Eri)

Samples	**α-Amylase inhibitory activity (IC_{50} µg/ml)**
Bombyx mori (Nanglai)	98.1 ± 2.3^{b}
Samia ricini (Eri)	125 ± 3.8^{c}
Acarbose (+ control)	54.8 ± 1.3^{a}

Values are mean of three replicate determinations (n = 3) ± standard deviation. Mean values followed by different superscripts in a column are significantly different (P <0.05; ANOVA, followed by Dunnett's multiple comparison test).

α-Glucosidase inhibitory activity

α-Glucosidase is an intestinal enzyme that catalyzes the break of α-1.4-glycosidic bond in oligosaccharides into α-glucose molecules which can be absorbed by the intestine. Figure 3 showed that the extracts of Nanglai and Eri exhibited a dose-dependent inhibition of α-glucosidase. Interestingly, acarbose showed significant higher IC_{50} (125.9 µg/ml) than that both protein extracts as shown in Table 3. The IC_{50} values of Nanglai and Eri were 36.4 µg/ml and 23.9 µg/ml, respectively. Eri extracts exhibited the strongest inhibitory activity, with approximately 6-fold higher activity when compared to acarbose. Our result indicate that

protein extracts from Eri and Nanglai are as efficient as the drug, acarbose, in inhibiting α-amylase activity, and are potential candidate for development of anti-hyperglycemic formulation.

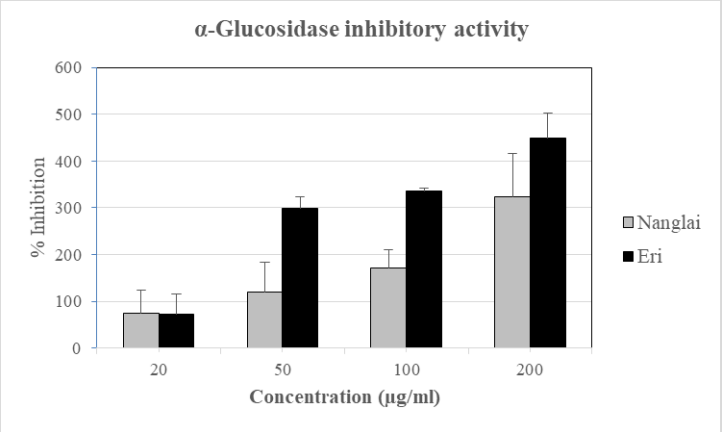

Figure 3. Inhibitory activity against α-Glucosidase of protein extracts from *Bombyx mori* (Nanglai) and wild silkworm, *Samia ricini* (Eri)

Table 3. α-Glucosidase inhibitory activity of protein extracts from *Bombyx mori* (Nanglai) and wild silkworm, *Samia ricini* (Eri)

Samples	**α-Glucosidase inhibitory activity (IC_{50} µg/ml)**
Bombyx mori (Nanglai)	36.4 ± 4.9^{b}
Samia ricini (Eri)	23.9 ± 4.3^{b}
Acarbose (+ control)	125.9 ± 10.4^{a}

Values are mean of three replicate determinations (n = 3) ± standard deviation. Mean values followed by different superscripts in a column are significantly different ($P < 0.05$; ANOVA, followed by Dunnett's multiple comparison test).

These results of α-amylase and α-glucosidase inhibitory activities are consistent with previous studies that extracts of silkworm pupa reduce blood glucose in diabetic rats, and decrease fat storage, this is considered to be effective in the inhibition of the metabolic syndrome[8].

Anti-Inflammation

Albumin denaturation inhibitory activity

Inflammation has been implicated in the pathogenesis of many diseases including arthritis, stroke, and cancer. Denaturation of albumin is the main cause of inflammation[20]. As part of the investigation on the mechanism of the anti-inflammatory activity, ability of protein extracts from Nanglai and Eri pupae to inhibit this protein denaturation was determined. Ibuprofen was used as a standard anti-inflammation drug. The inhibitory manner and IC_{50} results of the protein extracts are presented in Figure 4 and Table 4. Both protein extracts showed a dose-dependent manner of inhibitory activity with good anti-denaturation activity. The capacity of protein extracts from Nanglai and Eri to inhibit the denaturation of albumin was observed by 50% inhibition at a concentration of 113.6 µg/ml, and 138.8 µg/ml, respectively. These results showed the significant lower IC_{50} values than that Ibuprofen (IC50 = 164.3 µg/ml). Hence, the ability of Nanglai and Eri to inhibit the denaturation of protein signifies apparent potential for anti-inflammatory activity.

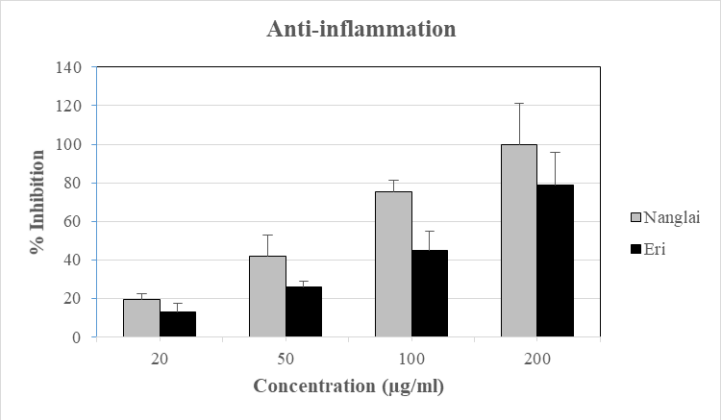

Figure 4. Inhibitory activity against albumin degradation of protein extracts from *Bombyx mori* (Nanglai) and wild silkworm, *Samia ricini* (Eri)

Table 4. Albumin degradation inhibitory activity of protein extracts from *Bombyx mori* (Nanglai) and wild silkworm, *Samia ricini* (Eri)

Samples	**Albumin degradation inhibitory activity (IC_{50} µg/ml)**
Bombyx mori (Nanglai)	113.6 ± 5.9^{b}
Samia ricini (Eri)	138.8 ± 4.7^{b}
Ibuprofen (+ control)	164.3 ± 5.3^{a}

Values are mean of three replicate determinations (n = 3) ± standard deviation. Mean values followed by different superscripts in a column are significantly different (P <0.05; ANOVA, followed by Dunnett's multiple comparison test).

Antioxidant activity

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. The main characteristic of an antioxidant is its ability to trap free radicals. Therefore, antioxidants play a potential role in preventing and repairing damages caused by oxidative stress. They may lead to better health and novel treatment approaches for many diseases, such as cancers, cardiovascular and neurodegenerative diseases.[21]

DPPH radical scavenging activity

Radical scavenging activities are very important to prevent the deleterious role of free radicals. DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants that can be quantitatively measured from the change in absorbance. The percentage of scavenging activity is then calculated. The results of DPPH radical scavenging assays of protein extracts from Nanglai and Eri pupae are given in Figure 5 and Table 5. The percentage of scavenging DPPH radicals at various concentrations of the extracts indicated that Nanglai and Eri had potential scavenging activity in a concentration-dependent manner, with a half maximal inhibitory concentration (IC_{50}) values of 416.6 µg/ml and 454.5 µg/ml, respectively which is significant lower than that of the reference standard, ascorbic acid. The highest DPPH scavenging activity was observed in Nanglai and it was approximately 4-fold higher activity when compared to ascorbic acid.

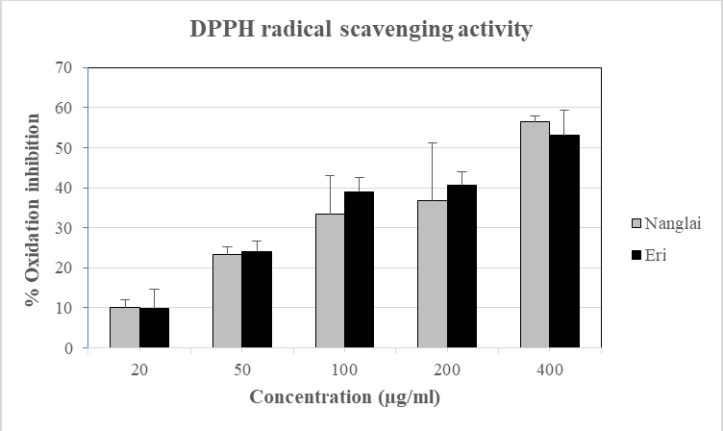

Figure 5. DPPH radical scavenging activity of protein extracts from *Bombyx mori* (Nanglai) and wild silkworm, *Samia ricini* (Eri)

Table 5. DPPH radical scavenging activity of protein extracts from *Bombyx mori* (Nanglai) and wild silkworm, *Samia ricini* (Eri)

Samples	**DPPH radical scavenging activity (EC_{50} µg/ml)**
Bombyx mori (Nanglai)	416.6 ± 14.4^{b}
Samia ricini (Eri)	454.5 ± 24.8^{b}
Ascorbic acid (+ control)	1679.5 ± 55.1^{a}

Values are mean of three replicate determinations (n = 3) ± standard deviation. Mean values followed by different superscripts in a column are significantly different ($P < 0.05$; ANOVA, followed by Dunnett's multiple comparison test).

Metal Chelating Activity

Iron can stimulate the lipid peroxidation by Fenton reactions, resulting in the generation of hydroxyl radicals. The assay performed to measure chelating ability of ferrous ion was based on the chelation of this ion with ferrozine to form ferrous-ferrozine complex. The chelating activity of protein extracts from silkworm pupae, *Bombyx mori* (Nanglai) and *Samia ricini* (Eri) were measured at the different concentrations and the results are presented in Figure 6 and Table 6. The results showed that protein extracts from Eri (EC_{50} = 14.4 µg/ml exhibited the highest ferrous ion chelating effects, whereas standard reference, ascorbic acid, (EC50 = 20.6 µg/ml) exhibited lowest ferrous ion chelating effects. Protein extracts from Nanglai showed slightly lower activity with EC50 values of 16.6 µg/ml than that Eri, but had stronger activity than that reference. Excess iron in the body is associated with iron-mediated oxidative stress, which increases the risks of neurodegenerative diseases, diabetes, cancer, and stroke. Hence, iron chelators are used as therapeutic agents in the management of iron-related diseases. Our results suggested that the protein extracts from Eri and Nanglai pupae had an effective chelating ability on ferrous ion. They also may be a potential candidate to develop as therapeutic agents.

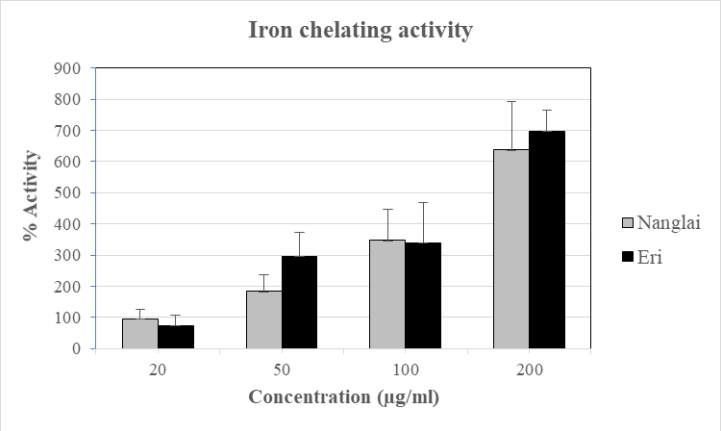

Figure 6. Iron chelating activity of protein extracts from *Bombyx mori* (Nanglai) and wild silkworm, *Samia ricini* (Eri)

Table 6. Iron chelating activity of protein extracts from *Bombyx mori* (Nanglai) and wild silkworm, *Samia ricini* (Eri)

Samples	**Iron chelating activity (EC_{50} µg/ml)**
Bombyx mori (Nanglai)	16.6 ± 3.1^a
Samia ricini (Eri)	14.4 ± 3.9^a
Ascorbic acid (+ control)	20.6 ± 2.2^a

Values are mean of three replicate determinations (n = 3) ± standard deviation. Mean values followed by different superscripts in a column are significantly different ($P < 0.05$; ANOVA, followed by Dunnett's multiple comparison test).

Conclusion

In this study, protein extracts from silkworm pupae, *Bombyx mori* (Nanglai) and *Samia ricini* (Eri) demonstrated that they exhibited excellent inhibitory potential against acetylcholinesterase, α-amylase, α-glucosidase, inflammation and radical scavenging activities in vitro. They also had the comparative abilities with commercial drugs (as positive control of each experiment). Nanglai displayed stronger albumin degradation, α-amylase and acetylcholinesterase inhibition, and higher DPPH radical scavenging activity, whereas Eri exhibited better anti-diabetic activity by inhibiting α-glucosidase and iron chelating activity. Hence, it may be concluded that protein extracts from both species have biological activities against most causes of NCDs, offering a possibility applying for the future therapeutic medicine for prevention and treatment of NCDs in the future. Further studies will be conducted on identification of bioactive compounds, molecular mechanisms involved in those inhibitory activities, determination of their efficacy by *in vivo* studies and demonstration of their safety and effectiveness in clinical trials.

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