Novel neuroprotective peptides derived from enzymatic hydrolysis of pea protein

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Research Article

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Novel neuroprotective peptides derived from enzymatic hydrolysis of pea protein

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Abstract

The aim of this study was to purify and identify neuroprotective peptides derived from Flavourzyme®-pea protein hydrolysate (FPPH). Cell viability of β-amyloid peptide ($A\beta_{1-42}$)-induced oxidative damage in SH-SY5Y cells was used to explore neuroprotective effects of pretreating the cells with or without hydrolysate and its fractions. The higher cell viability represents the better neuroprotective effect. The hydrolysate was sequentially purified by gel filtration (GF) chromatography, reversed-phase (RP) chromatography and cation-exchange (CE) chromatography to explore any fraction to increase neuroprotective effects. The peptides were identified using a liquid chromatography tandem mass spectrometer (LC/MS/MS). Three novel neuroprotective peptides were obtained, and the amino acid sequences were NKFGKFF, GGPFKSPF and RPVLGGSSTFPYP. The *in vitro* effect of

gastrointestinal proteases on neuroprotective effects of the three peptides were also investigated. The result suggested that the gastrointestinal protease did not affect neuroprotective effects of the three novel peptides, which reveal the potential to ameliorate disease caused by neurodegeneration.

Keywords: Pea protein, Neuroprotective peptides, Cell viability, $A\beta_{1-42}$, SH-SY5Y cells

Introduction

Ageing is the primary risk factor for most neurodegenerative diseases, including Alzheimer disease (AD) and Parkinson disease. One in ten individuals aged ≥ 65 years has AD and its prevalence continues to increase with increasing age (Hou et al., 2019). AD is characterized by the extracellular aggregation of amyloid-β peptide, intracellular inclusions of neurofibrillary tangles and hyperphosphorylation of Tau protein in neurons (Hyman et al., 2012). The brain of AD patients shows higher oxidative damage along with abnormal amyloid-β peptide accumulation and neurofibrillary tangles (Cassidy, et al., 2020). Memory and cognitive impairments/disorders are the main clinical symptoms of neurodegenerative diseases. The prevalence of such chronic conditions has increased over the past three decades. There is a

41 growing interest in the use of food-derived bioactive products as intervention
42 agents in functional foods and dietary supplements for the fights against these
43 human diseases (Wang, Waterhouse, Waterhouse, Zheng, Su, & Zhao, 2021).
44 Among many classes of compounds considers as neuroprotective agents,
45 peptides derived from natural materials or their synthetic analogs are good
46 candidates (Perlikowska, 2021).

47 Pea protein is a mainstream ingredient which can enhance the nutritional
48 and technological properties to formulate or reformulate innovative food and
49 beverages (Boukid, Rosell & Gastellari, 2021). Several enzymatic hydrolysis
50 of pea protein to release peptides with bioactive activities are also reported,
51 such as antioxidant activity (Girgih, Chao, Lin, He, Jung, & Aluko, 2015;
52 Ding, et al., 2020), angiotensin I-converting enzyme inhibitory activity
53 (Barbana & Boye, 2010) and angiotensin converting enzyme II up-regulating
54 activity (Liao, Fan, Liu, & Wu, 2019). However, considering the large variety
55 of peptides regarding size and amino acids sequences many of them still is
56 unexplored. According to recent review of food derived bioactive peptides on
57 neuroprotective effect, pea peptides have not yet been investigated (Wang,
58 Waterhouse, Waterhouse, Zheng, Su, & Zhao, 2021).

59 $A\beta_{1-42}$ is widely applied to induce or as a toxic agent to establish both *in*
60 *vitro* (Alghazwi, Charoensiddhi, Smid, & Zhang, 2020; Rajput, Nirmal,
61 Rathore, & Dahima, 2020; Sirin & Aslim, 2021) and *in vivo* (Zhang et al.,
62 2019; Giacomeli, Izoton, Santos, Boeira, Jesse, & Haas, 2019) models for
63 investigating neuroprotective effects. $A\beta_{1-42}$-induced oxidative damage in
64 SH-SY5Y cells, human neuroblastoma, is one of the applications. It causes the
65 activation of several biochemical pathogenic mediators such as oxidative
66 stress, which activate the pathogenesis of AD (Sirin & Aslim, 2021). To study
67 neuroprotective effects, the cells are pretreated or preincubated with different
68 concentrations of target samples, and then subsequently expose to 1-10 μM of
69 $A\beta_{1-42}$, which induce oxidative damage to the cells (Rajput, Nirmal, Rathore,
70 & Dahima, 2020; Sirin & Aslim, 2021).
71 The objective of this study was to isolate neuroprotective peptides from
72 enzymatic pea protein hydrolysate through $A\beta_{1-42}$-induced oxidative damage
73 in SH-SY5Y cells as well as to identify several new neuroprotective peptides.
74

75 # Materials and methods

76 ## *Chemicals*

77 Pea protein (PP) was obtained from JIU PU Enterprise Corporation (Taichung,

78 Taiwan), and its crude protein content was 80% by the Kjeldahl method.
79 Flavourzyme® 1000 MG (1000 unit/g), Sodium carbonate, dexamethasone
80 and insulin were purchased from Sigma-Aldrich (Missouri, USA). Standards
81 including cytochrome c, aprotinin, gastrin I, substance P and glycine with a
82 retention time of 13.0, 17.1, 20.0, 25.8 and 28.8 min in the HPSEC (high-
83 performance size-exclusion chromatography), respectively, were purchased
84 from Merck (Darmdstadt, Germany) or Sigma-Aldrich (Missouri, USA).
85 Minimum essential medium (MEM), fetal bovine serum (FBS), trypsin-EDTA
86 and F-12 nutrient mixture for human neuroblastoma SH-SY5Y cell were
87 purchased from GIBCO (Grand Island, NY, USA). Retinoic acid and β-
88 amyloid ($A\beta_{1-42}$) were obtained from Cayman Chemical Com. (Michigan,
89 USA).
90
91 ***Preparation of neuroprotective hydrolysate***
92 Neuroprotective hydrolysate was prepared according to our preliminary study.
93 Briefly, 6 % (w/v) PP and 1 % (w of enzyme/w of PP) Flavourzyme® were
94 mixed at 50°C for 6 h to produce hydrolysate with the highest neuroprotective
95 effect in Aβ-induced oxidative damage in SH-SY5Y cells (ATCC®, Missouri,
96 USA). Parts of FPPH was freeze-dried to assay for neuroprotective effect in

terms of cell viability of the cells. The rest of sample was subjected to centrifugation at 8000 × g in a micro-centrifuge for 15 min. The supernatant was further fractionated by gel filtration chromatography.

Purification of neuroprotective peptide

Neuroprotective hydrolysate was loaded on a Superdex™ peptide 10/300GL column (10 mm x 300 mm) (Illinois, USA) equilibrated with 0.02 M sodium phosphate (pH 7.2) at a flow rate of 0.5 mL/min. Fractions were collected every 0.5 min with a fraction collector. Elution curves were obtained by measuring absorbance at 214 nm. The fractions that showed the highest neuroprotective effect in the cells were collected and further purified on InerSustain® reverse-phase (RP) C18 column (10 mm x 250 mm)(GL Sciences, Tokyo, Japan). The fractions were eluted with 0.1% trifluoroacetic acid at a flow rate of 1 mL/min. The eluate was detected at 214 nm. The highest active fractions were collected and further purified on Luna® 5 μm SCX 100 Å column (10 mm x 250 mm)(Phenomenex, Madrid, USA) with a linear gradient of 10 mM KH_2PO_4 25% ACN (pH 3.0)(0-40%) containing 350 mM KCl at a flow of 1 mL/min.

116 ***Cell culture and treatment***

117 Human neuroblastoma SH-SY5Y cells were seeded in 96-well plate at a final
118 concentration of 2.5 $\times 10^4$ cells/well in MEM/F-12 supplemented with 10%
119 FBS and 2mM L-glutamine in a humid atmosphere of 5% CO2 and 95% air
120 at 37 C. To induce differentiation, post-confluent SH-SY5Y cells were
121 stimulated for 48 h in MEM/F-12 containing 10 μM retinoic acid. The medium
122 was changed every 2 days. After 7 days, cells were pretreated with or without
123 FPPH and its fractions at indicated concentration for 24 h. And then MEM/F-
124 12 containing 10 μM $A\beta_{1-42}$ was added to induce oxidative damage in SH-
125 SY5Y cells for another 24 h. The neuroprotective effects of FPPH and its
126 fractions was thus determined in cell viability assay.

127

128 ***Cell viability assay***

129 Neuroprotective effects in terms of cell viability were determined. Cell
130 viability of SH-SY5Y cells (control, CON) as well as $A\beta_{1-42}$-induce oxidative
131 damage cells pretreated with or without FPPH and its fractions were
132 investigated by using Alamar Blue™ (AB) assay (Thermo Fisher Scientific,
133 Waltham, USA). The AB assay was carried out according to manufacturer's
134 instructions. Briefly, control medium was removed. The cells were rinsed with

PBS and 2 mL of an 5% AB solution prepared in fresh medium were added to each well. Following 3 h incubation, AB was reduced to pink color and measured at 570 nm using a Thermo Scientific microplate reader. The absorbance at 570 nm of the sample (A_{sample}) and control ($A_{control}$) against the reagent blank was measured. Cell viability was calculated as

$$\text{Cell viability (\%)} = (A_{sample} / A_{control}) \times 100 \quad (1)$$

Amino acid sequence of peptide

Amino acid sequence and molecular mass of each purified peptide were determined using a liquid chromatography tandem mass spectrometer (LC/MS/MS) of Finnigan LTQ XL (Thermo Electron Co., Massachusetts, USA) coupled with an electro-spray ionization source.

Effect of gastrointestinal proteases digestion on neuroprotective effect

The stability against in vitro gastric proteases was assessed as described by Wu and Ding (2002) with some modification. One percent (w/v) of peptide was treated in 0.1 M KCl-HCl buffer (pH 2.0) with pepsin for 4 h in a water bath at 37 ℃. The reaction was stopped by boiling in a water bath for 15 min and neutralized to pH 7.0 with the addition of 2 N NaOH solution. One

154 milliliter of neutralized suspension was centrifuged (10,000×g, 40 min) and
155 the supernatant was then used for neuroprotective effect determination. The
156 remaining neutralized suspension was digested further by 2 % pancreatin at
157 37℃ for 4 h. The enzyme was inactivated by boiling for 15 min followed by
158 centrifugation (10,000×g, 40 min). The supernatant was then used to
159 determine the neuroprotective effects.
160
161 ***Statistical analysis***
162 All experiments were carried out three times, and the data were expressed as the
163 mean ± SD. Statistical analysis was performed by one-way analysis of variants
164 (SAS 9.4). Differences between means were analyzed by Duncan's multiple
165 range test. Data were considered statistically significant at $p < 0.05$.
166
167 # Results
168 **Isolation of neuroprotective effect fractions from FPPH**
169 Neuroprotective effects of pretreating SY5Y cells with or without FPPH under
170 different concentrations before $A\beta_{1-42}$-induced oxidative damage in terms of
171 cell viability were determined (Fig. 1). The higher cell viability represents the
172 better neuroprotective effect. The result showed that 10 μM $A\beta_{1-42}$

significantly decreased the cell viability by 40% and successfully induced damage of SH-SY5Y cells as compared with control (CON). Increase of cell viability was not significantly observed as pretreating the cells with FPPH less than 250 ppm until those greater than 500 ppm. Subsequently gel filtration chromatography of FPPH resulted in six fractions of GF-1, GF-2, GF-3, GF-4, GF-5 and GF-6 (Fig. 2a). The neuroprotective effect of pretreating SH-SY5Y cells with FPPH and those six fractions before $A\beta_{1-42}$-induced oxidative damage, respectively, as presented on Fig. 2b. The result indicated that all the fractions still had significant neuroprotective effects in the cells ($p < 0.05$). Among them GF-3 had highest neuroprotective effect and significant increase of cell viability from 55.0% to 79.3% ($p < 0.05$) after gel filtration of FPPH. GF-3 was subjected to further fractionate using InerSustain® RP-HPLC C18 column as depicted in Fig. 3a and two fractions were collected. RP2 was identified as the most effect fraction to increase cell viability up to 89.9% against $A\beta_{1-42}$-induced oxidative damage in SH-SY5Y cells (Fig. 3b), and further fractionated by Luna® SCX cation-exchange column to obtain two fractions (Fig. 4a). Only CE-2 could further significantly increase cell viability up to 91.4% ($p < 0.05$), which was identified as the most potent fraction of neuroprotective effect in the cells (Fig. 4b).

Identification of neuroprotective effect peptides

Peptide sequences of CE-2 was analyzed by LC/MS/MS. There were 3 peptide sequences identified including NKFGKFF, GGPFKSPF and RPVLGGSSTFPYP (Fig. 5). To ensure the neuroprotective effect of each purified peptide against $A\beta_{1-42}$-induced oxidative damage in SH-SY5Y cells, the three peptides were synthesized and their effects were further investigated. Fig. 6 compared neuroprotective effects of pretreating SH-SY5Y cells with or without 500 ppm FPPH and 0.5-5 ppm of each synthetic peptide before $A\beta_{1-42}$-induced oxidative damage in terms of cell viability. Pretreating the cells with FPPH and all three synthetic peptides showed significantly higher neuroprotective effects as compared to without pretreating in Aβ groups ($p < 0.05$). Both NKFGKFF and GGPFKSPF had significantly higher neuroprotective effects than FPPH and RPVLGGSSTFPYP ($p < 0.05$) regardless of concentrations. However, there were no significant difference of the effects between NKFGKFF and GGPFKSPF at any concentration. Neuroprotective effects of NKFGKFF and GGPFKSPF showed dose response after pretreating the cells with concentration less than 1 ppm, respectively, whereas no significant difference after those with concentration greater than 1

ppm. The above results indicated that NKFGKFF and GGPFKSPF proved to be the major peptides responsible for neuroprotective effects in FPPH.

Effect of gastrointestinal proteases digestion on neuroprotective effects

Comparison of neuroprotective effects of pretreating the cells with or without 1 ppm of each synthetic peptide followed by digestion using gastrointestinal proteases on cell viability before $A\beta_{1-42}$-induced oxidative damage were investigated (Fig. 7). All synthetic peptides showed significant neuroprotective effect before and after digested by gastrointestinal proteases ($p < 0.05$). Both NKFGKFF and GGPFKSPF showed little effect on cell viability after pepsin digestion as well as after pepsin and pancreatin digestion. Although the cell viability of RPVLGGSSTFPYP significantly decreased from 72.5% to 61.5% after pepsin digestion, a stable neuroprotective effect of RPVLGGSSTFPYP was still observed after pepsin and pancreatin digestion. The above result suggested that gastrointestinal protease had little effect on the neuroprotective effect of the three synthetic peptides.

Discussion

The peptides with neuroprotective effects were purified from FPPH

230 throughout a three-step purification procedure. The bioactive peptides were
231 identified to be NKFGKFF, GGPFKSPF and RPVLGGSSTFPYP. Pretreating
232 SH-SY5Y cells with functional ingredients before $A\beta_{1-42}$-induced oxidative
233 damage has been widely applied as model for neuroprotective effect
234 measurement in several studies (Rajput, Nirmal, Rathore, & Dahima, 2020;
235 Sirin & Aslim, 2021). In our study, pretreating the cells with any of the three
236 peptides significantly showed increase of cell viability as compared to without
237 pretreating. To protect the cells from oxidative damage by $A\beta_{1-42}$ becomes a
238 potential to prevent oxidative stress mediated AD (Sirin and Aslim, 2021).
239 Since these peptides with neuroprotective effects have not been reported yet
240 to our best knowledge, they are thought to be novel active peptides for
241 neuroprotective effects.

242 Several reports have highlighted that peptides with antioxidant activities
243 could reveal and apply them as potential therapeutic agents to improve the
244 health benefits, especially in the context of neuroprotection (Sarmadi &
245 Ismaila, 2010; Cicero, Foppoli & Colletti, 2017; Nwachukwu & Aluko, 2019).
246 Since pea protein hydrolysates and their peptides showed a lot of antioxidant
247 activities (Pownall, Udenigwe & Aluko, 2011; Girgih, Chao, Lin, He, Jung, &
248 Aluko, 2015; Din et al., 2020), they might have the potential to reveal

249 neuroprotective effects. Furthermore, $A\beta_{1-42}$-induced oxidative damage in
250 SH-SY5Y cells was applied to evaluate the neuroprotective effects in this
251 study, which closely correlated to the antioxidant activities of pea protein
252 hydrolysates. It has been postulated that peptides containing amino acid
253 residues such as Asp, Pro, Trp, Tyr, Met, Cys, Leu, Arg, Ala, Phe and His show
254 higher antioxidant activities (Liu et al., 2018). Since NKFGKFF contains three
255 Phe (F), GGPFKSPF contains two Pro (P) and two Phe as well as
256 RPVLGGSSTFPYP contains Tyr (Y), Leu (L), Arg (R), Phe and three Pro, all
257 these peptides are expected to reveal considerable antioxidant activities. In
258 general, hydrophobic amino acids such as Leu or Val (V) and aromatic amino
259 acid such as Phe or Tyr are able to enhance the radical scavenging abilities of
260 peptides (Sarmadi & Ismaila, 2010; Liu et al., 2018). Moreover, amino acids
261 with aromatic residues donate protons to electron-deficient radicals (Liu et al.,
262 2018). The presence of Tyr contributes antioxidant activity just like many
263 other phenolic compounds to donate protons to radicals via hydroxy group on
264 the benzene ring. Unlike Tyr, Phe contributes antioxidant activity due to its
265 easily abstracted by free radical hydrogen in the methyl group (Power,
266 Jakeman & FitzGerald, 2013). Among many hydrolysates and peptides
267 derived from natural materials exerted neuroprotective active by suppressing

free radicals and oxidative stress markers (Lee & Hur, 2019).

Recently, peptides with neuroprotective activities have been reviewed and concluded that their amino acid compositions were related to neuroprotective activities. Eighty-eight peptides with neuroprotective activities reported in the literature were compared. The compositions of neuroprotective peptides showed that the percentage of the peptides containing hydrophobic, non-aromatic hydrophobic and aromatic hydrophobic amino acids reached 92%, 74% and 64%, respectively (Wang, Waterhouse, Waterhouse, Zheng, Su, & Zhao, 2021). In our finding, the three novel peptides were all containing hydrophobic amino acids. Among them NKFGKFF only had one type of hydrophobic and aromatic amino acid, Phe. Except Phe, both GGPFKSPF and RPVLGGSSTFPYP had Pro. Val and Leu were only found in RPVLGGSSTFPYP. The hydrophobicity of peptides enabled their penetration into the cells to reach and act on their specific targets, as well as increasing the solubility of the peptides in lipids facilitated in contact with hydrophobic radical species (Vo, Ryu & Kim, 2013; Perlikowska, 2021). Furthermore, basic amino acids found in 50% of the neuroprotective peptides also contributed to neuroprotective functions (Wang, Waterhouse,

286 Waterhouse, Zheng, Su, & Zhao, 2021). Basic amino acids, especially Lys (K)
287 and Arg (R), were found to reduce the acetylcholinesterase (AchEase) activity
288 via forming stable complexes with the peripheral anionic site of AchEase
289 (Havekes, Vecsey & Abel, 2012; Su et al., 2016). Due to AchEase activity,
290 the pathophysiology of AD was related to the reduction of the neurotransmitter
291 acetylcholine, a key signaling molecule that regulated memory and learning
292 functions in brain (Sussman et al., 1991; da Silva et al., 2011). As matter of
293 fact, GGPFKSPF and RPVLGGSSTFPYP containing Lys and Arg,
294 respectively, might contribute to their neuroprotective functions through
295 binging and inactivating AchEase.

296 Because of the increasingly strict ethics regulations on animal studies and
297 high costs of clinical trials, *in vitro* simulated gastrointestinal digestion model
298 has become a rapid and well-accepted approach to mimic the digestion process
299 of peptides prior to conducting *in vivo* studies (Wu and Ding, 2002). In this
300 study, the synthetic peptides were conducted for their effects in vitro by
301 treating it with gastrointestinal proteases, including pepsin and a combination
302 of pepsin and pancreatin, respectively. The results indicated that
303 gastrointestinal proteases had no significant effect ($p > 0.05$) on the
304 neuroprotective effects of NKFGKFF and GGPFKSPF. Significant decrease

of cell viability was observed for RPVLGGSSTFPYP after pepsin digestion, while significant increase of its cell viability was recovered after pepsin and pancreatin digestion.

In summary, three novel peptides, NKFGKFF, GGPFKSPF and RPVLGGSSTFPYP, were successfully purified and identified from the Flavourzyme®-pea protein hydrolysate. Pretreating SH-SY 5Y cells with any of these peptides exhibited a notable neuroprotective effect as compared to the oxidative damage Aβ group. Simulated gastrointestinal digestion did not affect their neuroprotective effect, which make these peptides have the potential to act as supplementary food ingredients for neurodegenerative diseases such as AD. In order to further confirm the identified peptides with neuroprotective effects, *in vivo* tests are needed.

Abbreviations

PP: Pea protein; FPPH: Flavourzyme®-pea protein hydrolysate; $A\beta_{1-42}$: β-amyloid peptide; GF: gel filtration; RP: reversed-phase; CE: cation-exchange; LC/MS/MS: liquid chromatography tandem mass spectrometer; AD: Alzheimer disease; MEM: Minimum essential medium; FBS: fetal bovine serum; AB: Alamar Blue™; CON: control.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The authors approved the consent for publishing the manuscript.

Availability of data and materials

The data generated and analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

Wen-Dee Chiang: Super-vision, Conceptualization, Formal analysis, Investigation, Writing-review & editing. **Yun-Chi Hsu**: Methodology, Investigation, Data curation and analysis.

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469

Fig. 1 Neuroprotective effects of pretreating SH-SY5Y cells with or without FPPH under different concentrations before $A\beta_{1-42}$-induced oxidative damage in terms of cell viability. Date expressed as mean ± SD (n=3). Means with different letters represent significant differences ($p < 0.05$) by Duncan's multiple range test.

Fig. 2 **a** Gel filtration chromatography of FPPH and **b** neuroprotective effect of pretreating SH-SY5Y cells with or without the fractions obtained from FPPH before $A\beta_{1-42}$-induced oxidative damage. Bars represent means ± SD, n=3. Means with different letters represent significant differences ($p < 0.05$) by Duncan's multiple range test.

Fig. 3 **a** Reverse-phase chromatography and **b** neuroprotective effects of pretreating SH-SY5Y cells with or without the fractions of GF-3 obtained from gel filtration chromatography before $A\beta_{1-42}$-induced oxidative damage in terms of cell viability. Bars represent means ± SD, n=3. Means with different letters represent significant differences ($p < 0.05$) by Duncan's multiple range test.

Fig. 4 **a** Cation-exchange chromatography and **b** neuroprotective activity of pretreating SH-SY5Y cells with or without the fractions of RP-2 obtained from reverse-phase chromatography before $A\beta_{1-42}$-induced oxidative damage in terms of cell viability. Bars represent means ± SD, n=3. Means with different letters represent significant differences ($p < 0.05$) by Duncan's multiple range test.

Fig. 5 The mass spectrum of CE-2 obtained from the cation exchange chromatography.

Fig. 6 Comparison of neuroprotective effects of pretreating SH-SY5Y cells with or without 500 ppm FPPH and three synthetic peptides, NKFGKFF (NF), GGPFKSPF (GF) and RPVLGGSSTFPYP (RP), under different concentrations before $A\beta_{1-42}$-induced oxidative damage in terms of cell viability. Bars represent means ± SD, n=3. Means with different capital letters represent significant differences within peptides at same concentration ($p < 0.05$) and different lowercase letters represent significant differences within different concentrations of the same peptide ($p < 0.05$).

Fig. 7 Comparison of neuroprotective effects of pretreating SH-SY5Y cells with or without 1 ppm of NKFGKFF (NF), GGPFKSPF (GF) and RPVLGGSSTFPYP (RP) following digested by gastrointestinal proteases, respectively, before $A\beta_{1-42}$-induced oxidative damage in terms of cell viability. Bars represent means ± SD, n=3. Means with different capital letters represent significant differences within treatment at the same peptide ($p < 0.05$) and different lowercase letters represent significant differences within different peptides of the same treatment ($p < 0.05$).

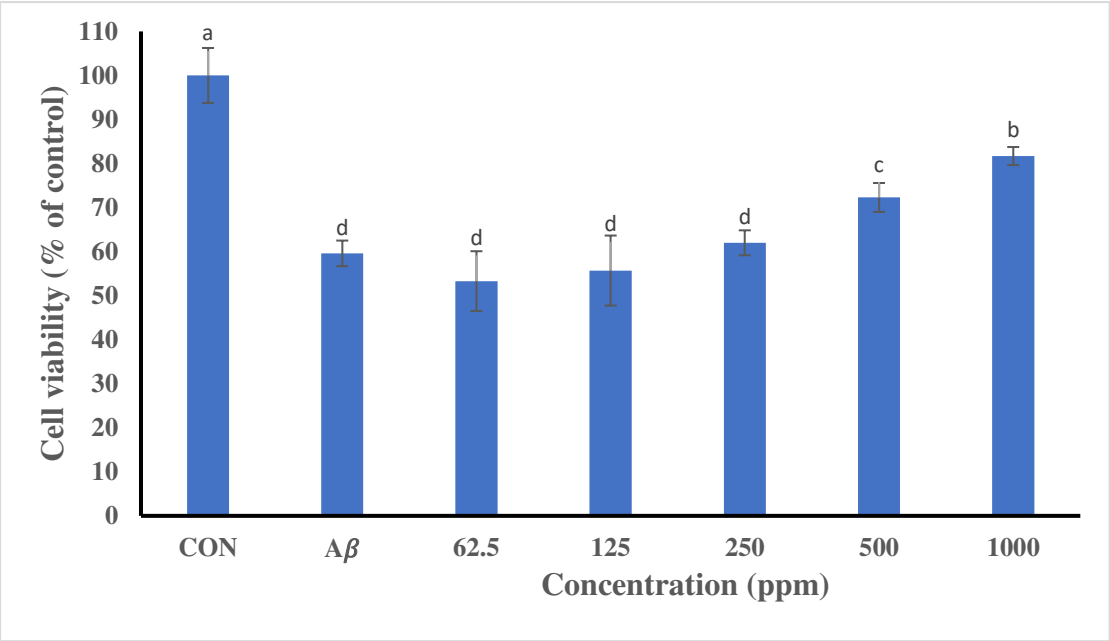
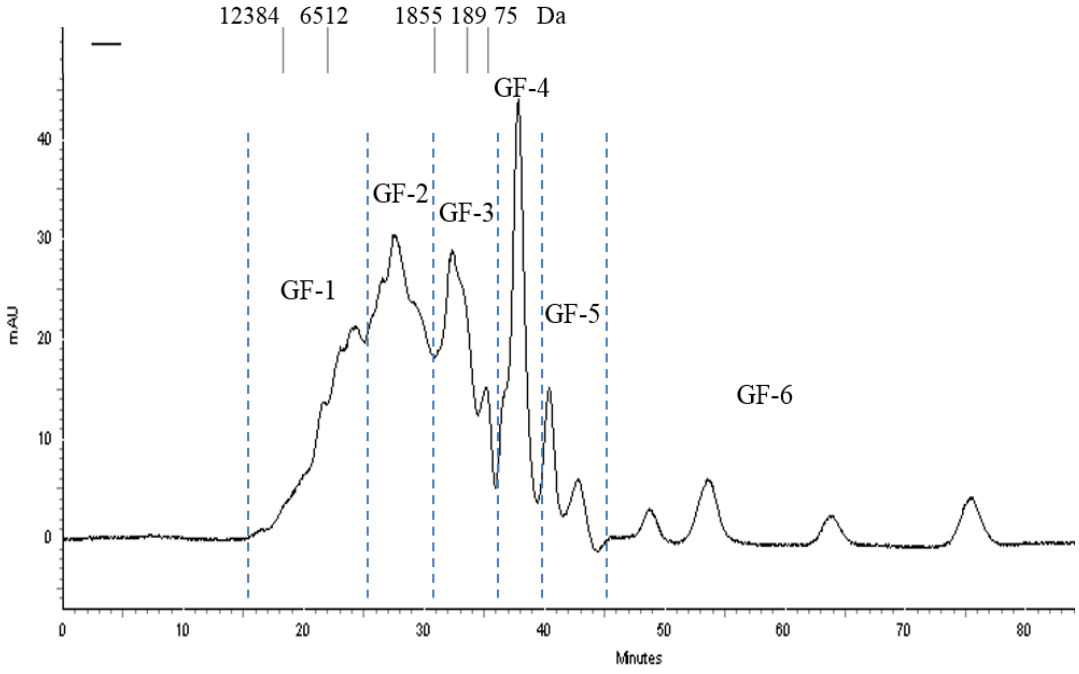
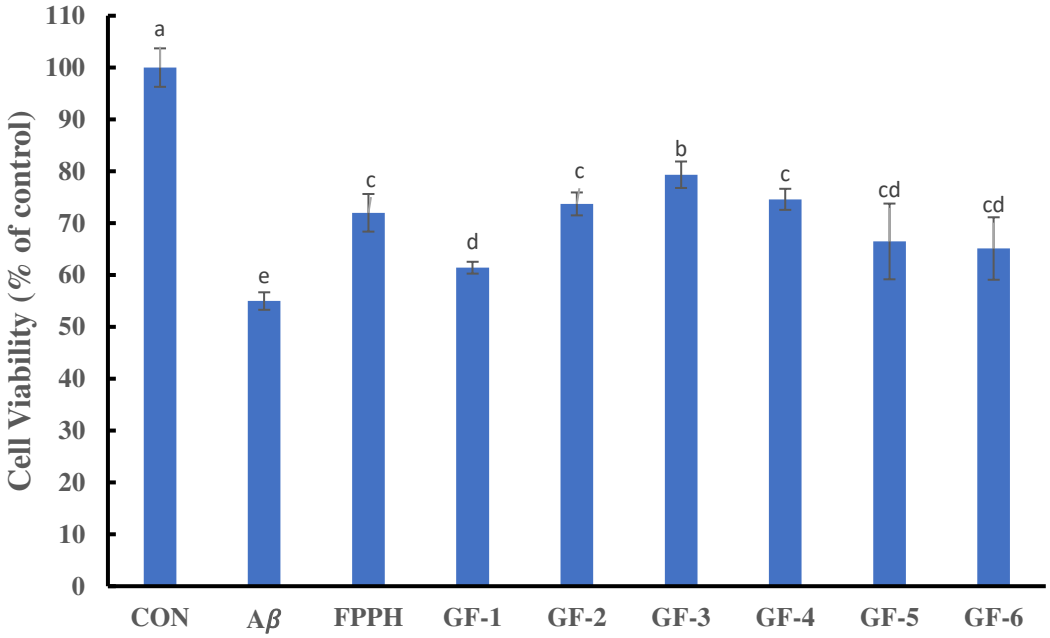

Cell viability (% of control)
110
100
90
80
70
60
50
40
30
20
10
0
a
d
d
d
d
c
b
CON
Aβ
62.5
125
250
500
1000
Concentration (ppm)

Fig. 1

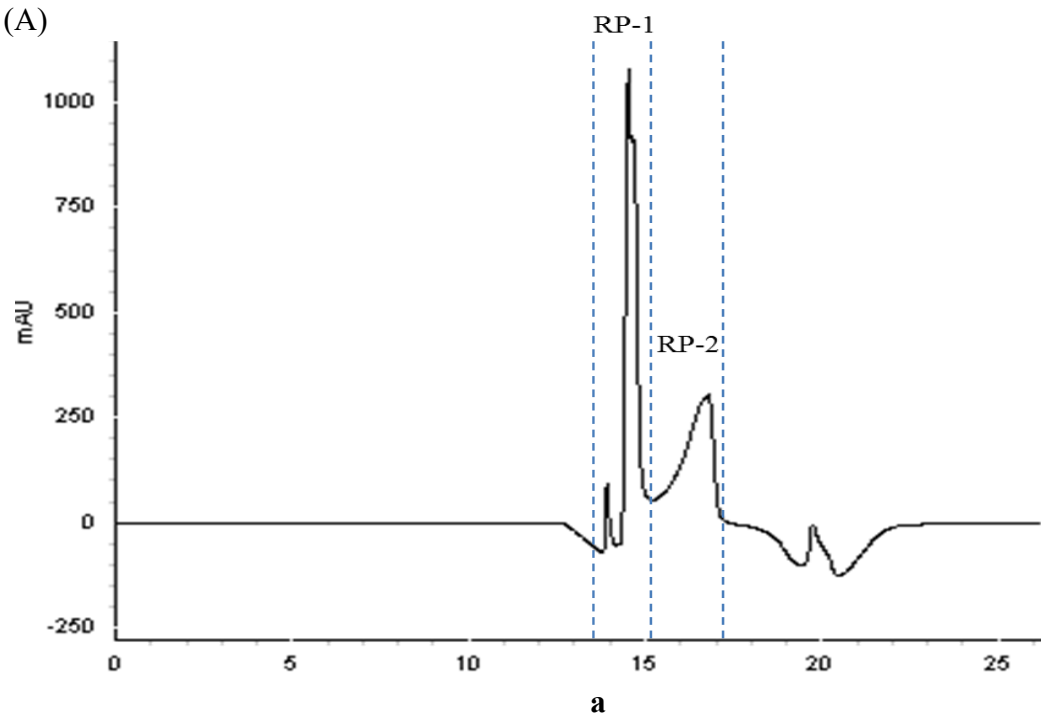

12384 6512 1855 189 75 Da
GF-1
GF-2
GF-3
GF-4
GF-5
GF-6
mAU
Minutes

a

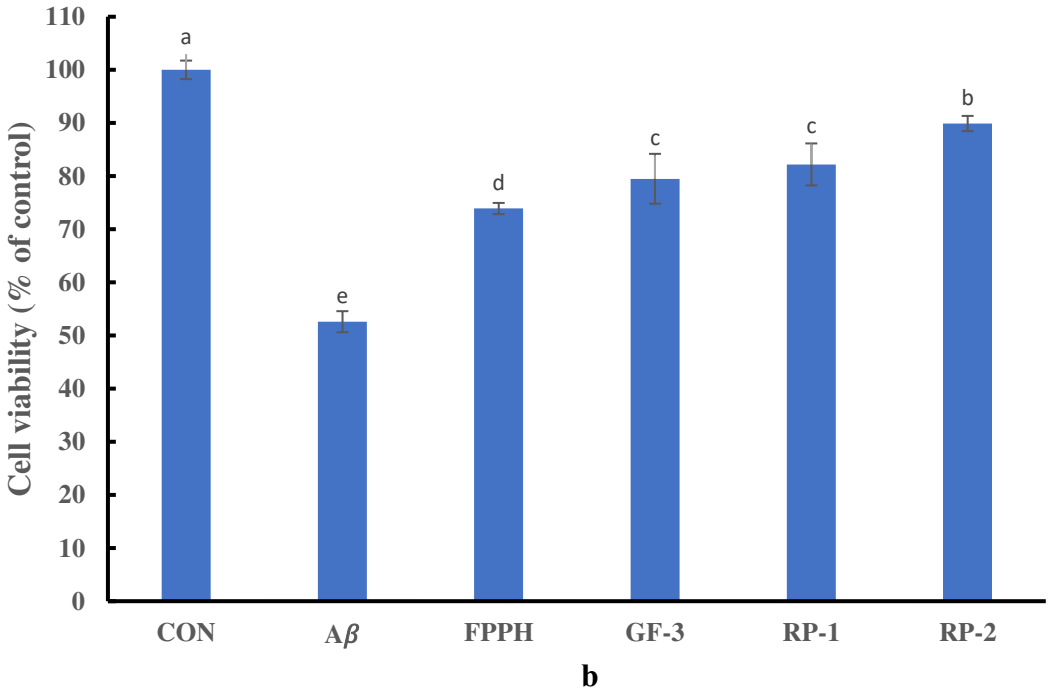

Cell Viability (% of control)
CON
Aβ
FPPH
GF-1
GF-2
GF-3
GF-4
GF-5
GF-6

b

Fig. 2

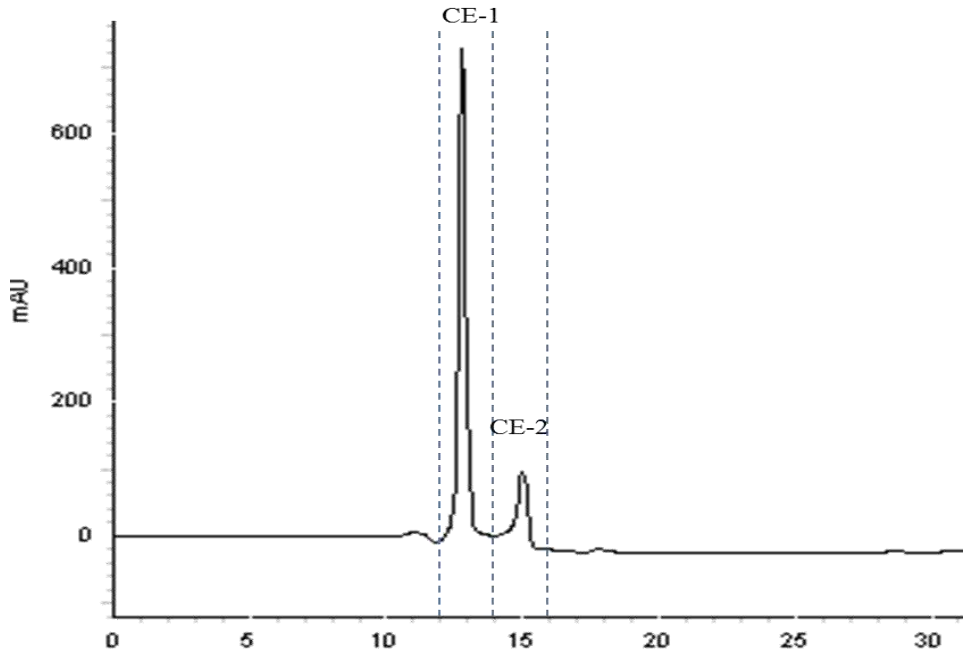

(A)
RP-1
RP-2
mAU
1000
750
500
250
0
-250
0
5
10
15
20
25

a

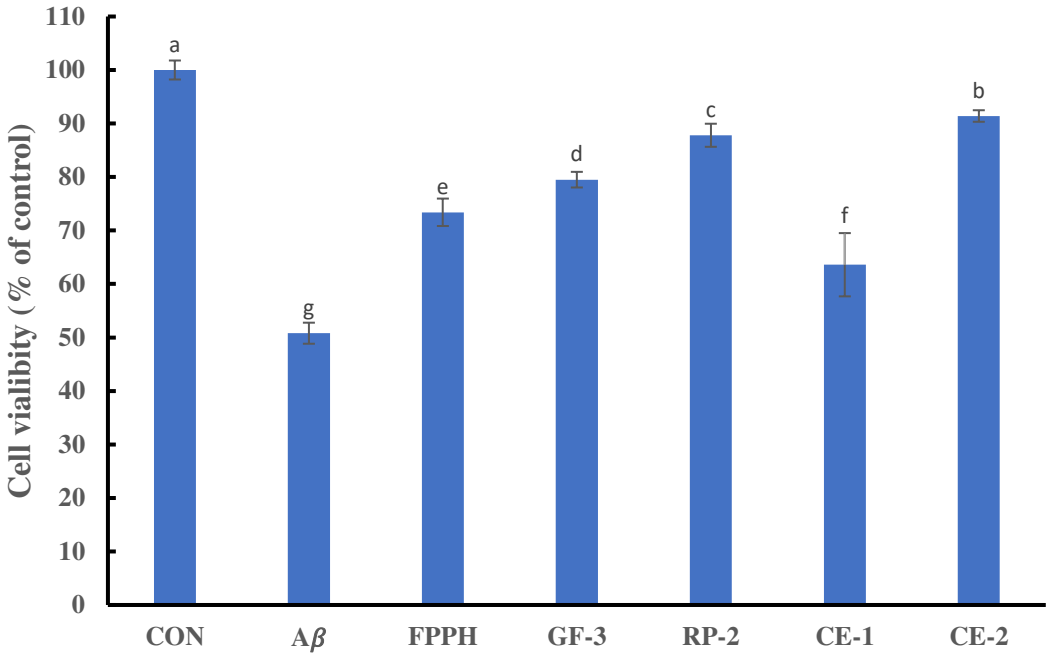

Cell viability (% of control)
110
100
90
80
70
60
50
40
30
20
10
0
a
e
d
c
c
b
CON
Aβ
FPPH
GF-3
RP-1
RP-2

b

Fig. 3

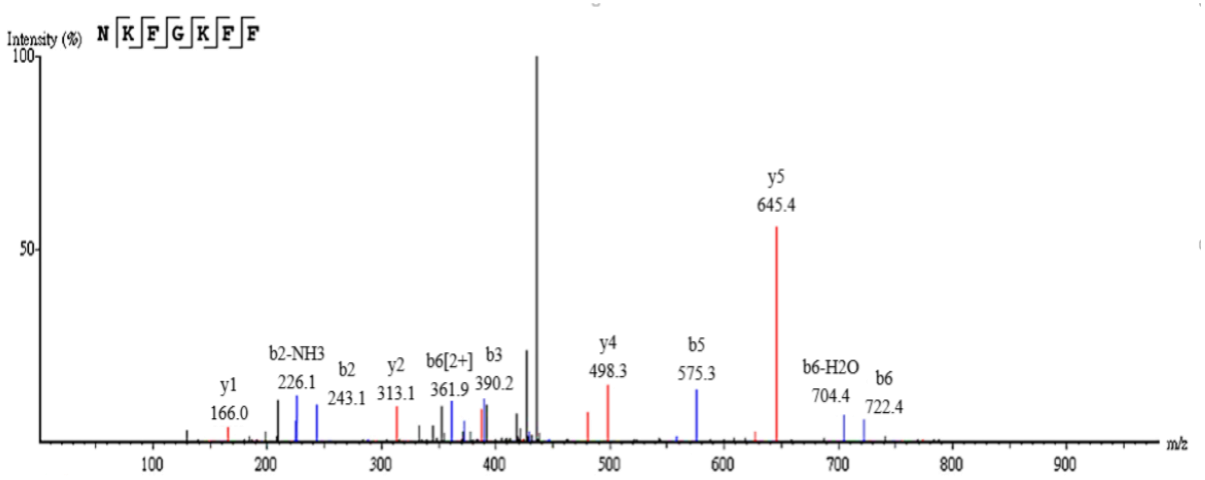

CE-1
CE-2
mAU
600
400
200
0
0
5
10
15
20
25
30

a


Cell viability (% of control)
110
100
90
80
70
60
50
40
30
20
10
0
a
g
e
d
c
f
b
CON
Aβ
FPPH
GF-3
RP-2
CE-1
CE-2

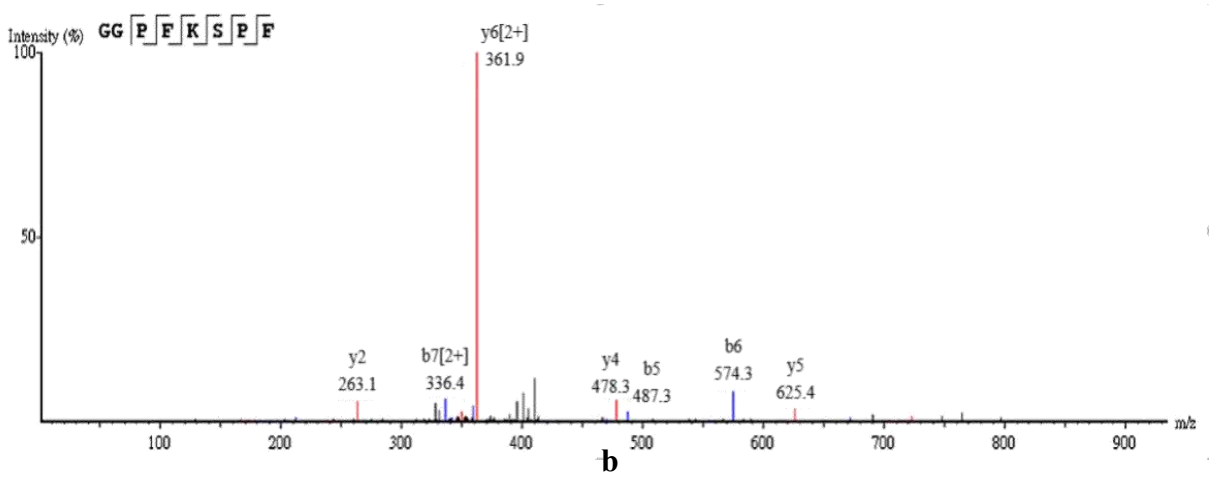
b

Fig. 4



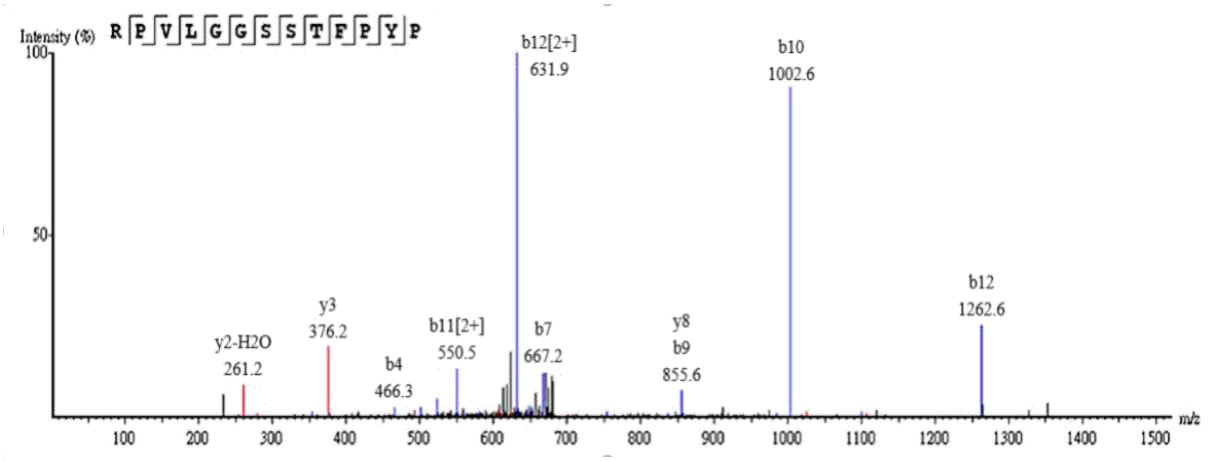
538
539 **a**

540



541 **b**

542



543

544 **c**

545 Fig. 5

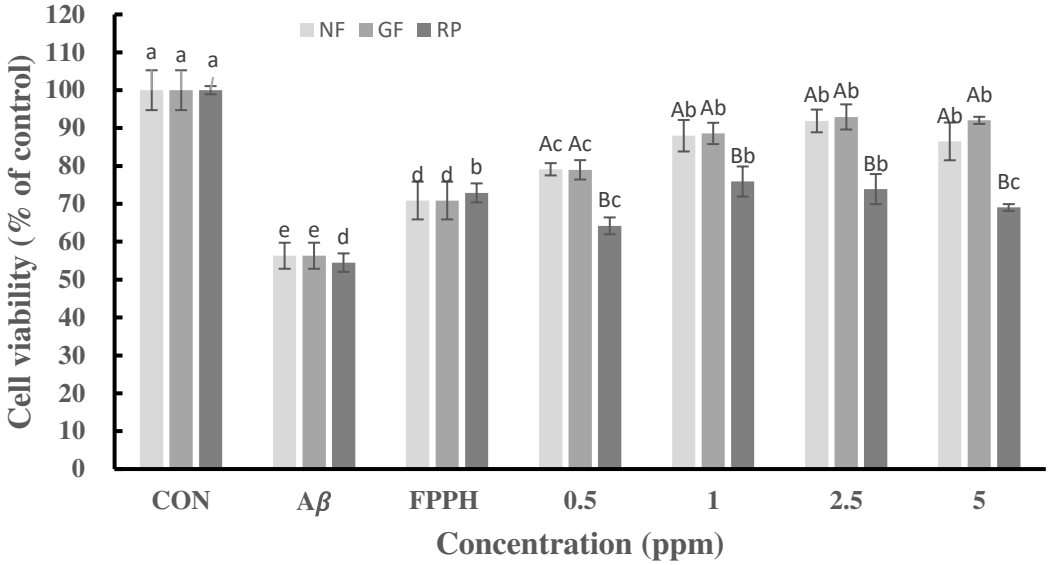

NF GF RP
Cell viability (% of control)
120
110
100
90
80
70
60
50
40
30
20
10
0
a a a
e e d
d d b
Ac Ac Bc
Ab Ab Bb
Ab Ab Bb
Ab Ab Bc
CON
Aβ
FPPH
0.5
1
2.5
5
Concentration (ppm)

Fig. 6

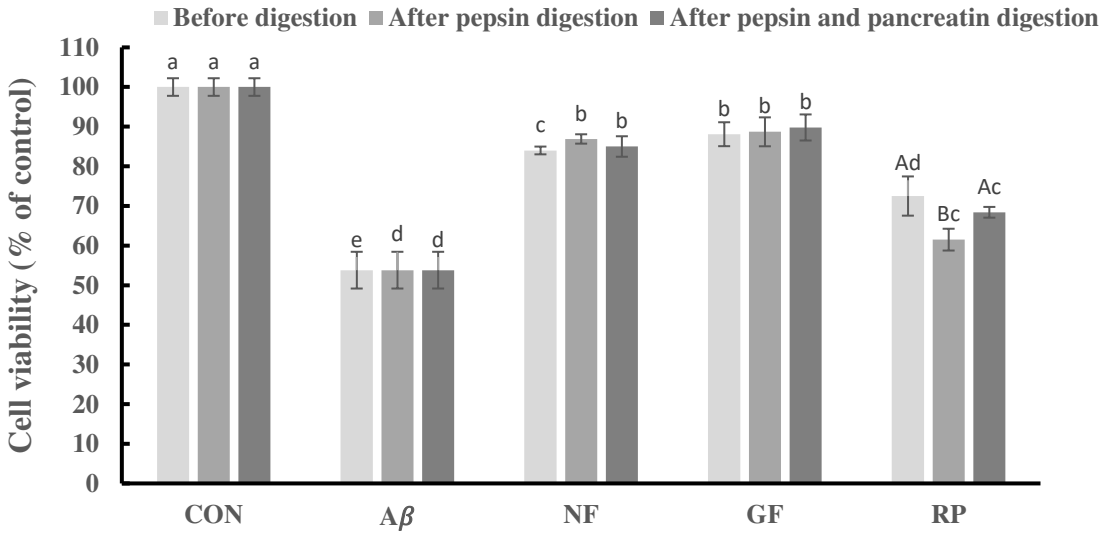

Before digestion
After pepsin digestion
After pepsin and pancreatin digestion
Cell viability (% of control)
110
100
90
80
70
60
50
40
30
20
10
0
a a a
e d d
c b b
b b b
Ad Bc Ac
CON
Aβ
NF
GF
RP

Fig. 7

Supplementary Files

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