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**Large-leaf yellow tea attenuates high glucose-induced
vascular endothelial cells injury by up-regulating autophagy
and down-regulating oxidative stress**

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Dear Editor,

Please find the enclosed manuscript entitled **“Large-leaf yellow tea attenuates high glucose-induced vascular endothelial cells injury by up-regulating autophagy and down-regulating oxidative stress”** submitted publication in **Food & Function**.

The work was original research and has not been submitted for publication, in whole or in part. The manuscript has been approved for publication by all authors.

Vascular endothelial cells injury induced by high glucose (HG) plays an important role in the occurrence and development of diabetic vascular complications. Yellow tea, a lightly fermented tea, has a protective effect on vascular endothelial cells. However, its molecular mechanism remains unclear. In this study, effect of the n-butanol fraction of Huoshan large-leaf yellow tea extract (HLYTBE) on vascular endothelial injury was investigated using human umbilical vein endothelial cells (HUVECs) and diabetic mice. The protection of HUVECs against injury induced by HG was first investigated. Our results showed that HLYTBE significantly reduced the production of reactive oxygen species, increased superoxide dismutase and glutathione peroxidase activity, enhanced the production of reduced glutathione production, and decreased the level of oxidized glutathione. HLYTBE promoted autophagosome formation, increased the expression of microtubule-associated protein 1 light chain 3-II/I ratio and Beclin1 and Atg 5, and decreased the expression of p62. HLYTBE up-regulated p-AMPK and down-regulated p-mTOR levels, which was reversed by the AMPK inhibitor Compound C. Furthermore, HLYTBE reduced apoptosis which was attenuated by the autophagy inhibitor 3-methyladenine. *In vivo* studies showed that HLYTBE improved the impaired pyruvate tolerance, glucose tolerance, and insulin resistance; reduced the concentrations of blood glucose,

glycated serum protein, lipids, and 8-isomeric prostaglandin 2 α in serum; increased the anti-oxidase activity in serum; and alleviated pathological damage in the thoracic aorta of diabetic mice induced by high sucrose-high fat diet and streptozotocin. These results suggest that HLYTBE protects the vascular endothelium against hyperglycemia-induced injury by up-regulating autophagy via the AMPK/mTOR pathway and inhibiting oxidative stress. These also provide a theoretical basis of large yellow tea to prevent or alleviate diabetic vascular complications.

Here we declare that there is no interest conflict with this article.

Thank you very much for your consideration of this work!

Best regards,

Sincerely yours,

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Highlights

- HLYTBE reduces oxidative stress in HUVECs treated with high glucose (HG).
- HLYTBE inhibits apoptosis in HG-treated HUVECs dependent by promoting autophagy.
- HLYTBE up-regulates autophagy in HG-treated HUVECs via AMPK/mTOR pathway.
- HLYTBE ameliorates glycolipid metabolism, IR and oxidative stress in diabetic mice.
- HLYTBE attenuates apoptosis and pathological damage in the aorta of diabetic mice.

**Large-leaf yellow tea attenuates high glucose-induced vascular
endothelial cells injury by up-regulating autophagy and
down-regulating oxidative stress**

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ABSTRACT:

Vascular endothelial cells injury induced by high glucose (HG) plays an important role in the occurrence and development of diabetic vascular complications. Yellow tea, a lightly fermented tea, has a protective effect on vascular endothelial cells. However, molecular mechanisms underlying this effect are not clear. In this study, effect of the n-butanol fraction of Huoshan large-leaf yellow tea extract (HLYTBE) on vascular endothelial injury was investigated using human umbilical vein endothelial cells (HUVECs) and diabetic mice. The protection of HUVECs against injury induced by HG was first investigated. Our results showed that HLYTBE significantly reduced the production of reactive oxygen species, increased antioxidase activity (superoxide dismutase and glutathione peroxidase), enhanced the production of reduced glutathione production, and decreased the level of oxidized glutathione. HLYTBE promoted autophagosome formation, increased the expression of microtubule-associated protein 1 light chain 3-II/I ratio and Beclin1 and Atg 5, and decreased the expression of p62. HLYTBE up-regulated p-AMPK and down-regulated p-mTOR levels, which was reversed by the AMPK inhibitor Compound C. Furthermore, HLYTBE reduced apoptosis and cytochrome C expression, which was attenuated by the autophagy inhibitor 3-methyladenine. *In vivo* studies showed that HLYTBE improved the impaired pyruvate tolerance, glucose tolerance, and insulin resistance; reduced the concentrations of blood glucose, glycated serum protein, lipids, and 8-isomeric prostaglandin 2 α in serum; increased the antioxidase activity in serum; and alleviated pathological damage in the thoracic aorta of diabetic mice induced by high sucrose-high fat diet and streptozotocin. These results suggest that HLYTBE protects the vascular endothelium by up-regulating autophagy via the AMPK/mTOR pathway and inhibiting oxidative stress.

Keywords: Autophagy; Apoptosis; High glucose; Large-leaf yellow tea; Vascular endothelial injury

1. Introduction

Tea is a popular beverage around the world owing to its refreshing fragrance. With increase in the degree of fermentation, tea can be classified into six categories: green, white, yellow, oolong, black, and dark¹. The different degree of fermentation gives the unique colour and flavour to tea by changing the content of chemical components and their degree of oxidation². Yellow tea is a lightly fermented tea, which tastes fresher and mellower than other types of tea and has various pharmacological effects^{3,4}. Teng reported that yellow tea could significantly decrease the levels of low-density lipoprotein, total cholesterol (TC), triglycerides, and blood glucose and increase glucose tolerance in db/db mice⁵. These findings suggest that yellow tea may have health benefits on diabetes.

Diabetes is a kind of metabolic diseases characterized by hyperglycaemia over a prolonged period, among which more than 90% are non-insulin-dependent diabetes mellitus, also known as type 2 diabetes mellitus (T2DM). With the change in dietary conditions, the age of patients with T2DM is gradually younger⁶. Specifically, the morbidity and mortality of diabetic patients with cardiovascular diseases is significantly increased⁷.

In high sugar environment, the increased intracellular levels of reactive oxygen species (ROS) in vascular endothelial cells could lead to reduced bioactivity of nitric oxide, vascular inflammation, leukocyte adhesion, and diabetic vascular complications⁸. Apoptosis, a kind of programmed cell death, may be over-activated in a high glucose (HG) environment, resulting in injury to endothelial cells and

vascular diseases ⁹. Under normal or mild stress conditions, autophagy may serve as a cell survival mechanism and for maintaining homeostasis. The autophagy of human umbilical vein endothelial cells (HUVECs) is impaired in HG environment. The up-regulation of autophagy can reduce mitochondrial damage, the level of ROS, and apoptosis rate, thereby, exerting protective effects on HUVECs ¹⁰. Studies have shown that hyperglycaemia enhances the expression of mammalian target of rapamycin (mTOR) by inhibiting adenosine 5'-monophosphate activated protein kinase (AMPK), thereby, inhibiting autophagy in HUVECs. These observations suggest that AMPK/mTOR signalling pathway is closely related to endothelial cell injury ¹¹.

There are few reports about the efficacy of yellow tea in preventing and treating diabetic vascular complications. In previous studies it has been suggested that the polar ingredients in large yellow tea may contribute to its anti-hyperglycaemic effects ⁴. Therefore, in present study, we first explored the effects of an n-butanol fraction of Huoshan large-leaf yellow tea extract (HLYTBE) on HG-induced autophagy and apoptosis in endothelial cells. Furthermore, we investigated the protective effects of HLYTBE on glucose and lipid as well as on vascular injury in type 2 diabetic mice induced by high sucrose-high fat diet and low-dose streptozotocin (STZ).

2. Materials and methods

2.1. Reagents

Metformin (Met, $\geq 99.0\%$) was obtained from Shouguang Fukang Pharmaceutical Co

(Shouguang city, Shandong, China). Low glucose Dulbecco's modified eagle medium (L-DMEM) and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA). Antibodies for autophagy-related gene 5 (Atg 5), Beclin 1, voltage-dependent anion channel 1, and glyceraldehyde-3-phosphate dehydrogenase were purchased from Bioworld Technology (St. Paul, MN, USA). Antibodies for p62, microtubule-associate protein 1 light chain 3 (LC3) were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies for cleaved caspase-3, superoxide dismutase (SOD) 2 were purchased from Wan Lei Biotechnology (Shenyang, China). Antibody for cytochrome c (Cyt.c) was purchased from Proteintech (Chicago, IL, USA). The assay kits for ROS, reduced glutathione (GSH) and oxidized glutathione (GSSG), bicinchoninic acid (BCA) protein assay kit and Hoechst 33258 staining kit were bought from Beyotime Institute of Biotechnology (Shanghai, China). STZ was purchased from Sigma Chemical Co, Ltd. (St. Louis, MO, USA). The kits for measurement of total SOD and glutathione peroxidase (GSH-Px) activity, blood glucose, glycated serum protein (GSP), TC, and triglycerides were purchased from Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). The enzyme-linked immunosorbent assay (ELISA) kits for insulin and 8-isomeric prostaglandin 2α (8-iso-PGF 2α) were bought from Jiangsu Meimian Industrial Co., Ltd. (Zhangjiagang, Jiangsu, China). Non-esterified fatty acids (NEFA) ELISA kit was purchased from Anoric Biotechnology (Tianjin, China).

114 2.2. Plant materials

115 Large-leaf yellow tea made from the leaves of the tea plant (*Camellia sinensis*), is one

of the most famous traditional yellow teas in China. Huoshan large-leaf yellow tea (HLYT) was purchased from Huoshan County, Lu'an City, Anhui Province, China. Processing HLYT from the mechanically harvested mature fresh tea leaves with stems includes six steps. The three initial steps fixation, rolling, and the first drying deactivate the endogenous enzymes in fresh tea leaves and reduce the moisture to about 10%, which are similar to those steps in processing green tea. Subsequently, the yellowing step (light fermentation) is performed to pile the tea leaves for 7 days and second drying is conducted under 120 – 140 °C for several minutes. The last roasting step under a temperature of 140–150 °C leads to the brown color and lightly astringent taste of the final yellow tea product ³.

2.3. Extract preparation

Ground powder of HYL T (9 kg) was extracted with 70% aqueous acetone, yielding 1.65 kg of extract. The acetone extract was dissolved in 4 L water, and extracted with dichloromethane, ethyl acetate, and n-butanol (v/v, 1:1) four times, successively, through ultrasonic extraction thrice (2 h each time), within 12 h by ultrasonic extraction, yielding dichloromethane (700 g), ethyl acetate (625 g), n-butanol (HYLTBE, 120 g), and final water (100 g) fractions.

HYLTBE (0.1 g) was stored in 10 mL of 70% aqueous methanol. The samples were centrifuged at 10,000 rpm for 10 min, and then the supernatant was run through a 0.22 µm filter for the further ultra performance liquid chromatography (UPLC) analysis.

2.4. Analysis of chemical components in HYL TBE

The content of flavonoid glycosides and tea polyphenols in HYL TBE was measured using UPLC¹². The Waters ACQUITY UPLC H-Class UPLC system equipped with a 2489 UV detector and a Waters C18 column (2.1 × 150 mm, 1.7 μm, Waters, Ireland) was used. The column heater was set at 30 °C. The mobile phase consisted of 0.17% acetic acid water and acetonitrile, and the flow rate was 0.22 mL/min. The samples were detected at 350 nm and quantified according to the calibration curve obtained for flavonoid glycosides.

2.5. Cell culture and treatment

HUVECs were purchased from Shanghai Honsun Biological Technology Co. Ltd. (Shanghai, China). HUVECs were cultured in L-DMEM containing 10% (v/v) FBS, supplemented with 100 U/mL penicillin and 100 U/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO₂ and 95% air. Passages 3 to 15 of HUVECs were used for the described experiments.

2.6. Detection of ROS production in HUVECs

HUVECs (5 × 10⁴ cells/well) were seeded in 6-well plates and cultured for 24 h. Thereafter, the cells were co-cultured with HLYTBE (10, 30, 60 μg/mL) or Met 350 μM in the presence or absence of glucose (33 mM) for 48 h. The 2',7'-dichlorofluorescein diacetate (DCFH-DA) fluorescent probe was used to measure the intracellular ROS generation. The cells were then treated according to the manufacturer's instructions. Briefly, the cells were washed and incubated in dark with

DCFH-DA (10 μ M) at 37 °C for 20 min. After washing 3 times with cold phosphate buffer solution (PBS), the cells were viewed under a fluorescent microscope (Olympus IX81, Tokyo, Japan) at an excitation wavelength of 488 nm and an emission wavelength of 525 nm ^{13,14}. The fluorescence intensity was quantified by densitometry using the Image J software (National Institutes of Health).

2.7. Detection of antioxidase activity and GSH and GSSG content in HUVECs

HUVECs (5×10^4 cells/well) were seeded in 6-well plates and cultured for 24 h. Thereafter, the cells were co-cultured with HLYTBE (10, 30, 60 μ g/mL) or Met 350 μ M in the presence or absence of glucose (33 mM) for 48 h. Then, the cells were washed twice with cold PBS and removed from the plates. Then, the cells were completely disrupted with ultrasonication on ice and centrifuged at 12,000 rpm for 10 min at 4 °C. The cell supernatant was used for the assessment of total SOD and GSH-Px activity and GSH and GSSG levels strictly according to the manufacturer's instructions. The protein concentration was determined by the BCA protein assay.

2.8. Observation of autophagosomes by transmission electron microscopy (TEM)

HUVECs (5×10^4 cells/well) were co-cultured with HLYTBE (60 μ g/mL) or Met 350 μ M in the presence or absence of glucose (33 mM) for 48 h. Subsequently, the cells were washed twice with PBS and were immobilized with 5% glutaraldehyde. After dehydration, saturation, embedding, sectioning, and staining, the cells were observed by TEM (JEM-1010, JEOL, Japan).

2.9. Western blot analysis

After the treatment, cells were lysed with a lysis buffer. The protein concentration in the lysate was determined with a BCA protein assay kit. Equal amounts of proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and subsequently transferred to polyvinylidene fluoride membranes. The membranes were blocked for 2 h at room temperature in 5% non-fat milk dissolved in Tris-buffered saline containing 0.05% Tween 20. Subsequently, the membranes were incubated overnight with the primary antibody (1:1000 dilution) at 4 °C. Thereafter, the membranes were washed and incubated with the secondary antibody (1:5000 dilution) for 2 h at room temperature. Finally, the protein bands were detected using an enhanced chemiluminescence detection system and the images were analysed using the Image J software.

2.10. The protein expression levels of Cyt.c in mitochondria and cytoplasm

HUVECs (1×10^5 cells/flask) were seeded in 75 mL cell culture flask and cultured for 24 h. Thereafter, the cells were co-cultured with HLYTBE (10, 30, 60 $\mu\text{g/mL}$) or Met 350 μM in the presence or absence of glucose (33 mM) for 48 h. After digestion with 0.25% trypsin, cells were collected and centrifuged at 1000 rpm for 5 min for 2 times. Then, the cells were resuspended in the mitochondria isolation reagent. The isolation and lysis of mitochondria were carried out in strict accordance with the manufacturers' instructions. Protein concentration was determined with a BCA protein assay kit. The Cyt.c protein expression levels in mitochondria and cytoplasm were detected using western blot analysis as described above.

2.11. Cell apoptosis assay by Hoechst staining

After the treatment, HUVECs were washed twice with PBS and stained with Hoechst 33258. The morphology of apoptotic cells was detected using a fluorescence microscope¹⁵. The fluorescence intensity was quantified by densitometry using the Image J software.

2.12. Animals

Male C57BL/6J mice (17–19 g) were purchased from Comparative Medical Center of Yangzhou University (Yangzhou, China). The animals were maintained with free access to laboratory chow and tap water in plastic cages at a controlled temperature (23 ± 2 °C), under a 12 h light/dark cycle. The experimental process followed the regulations prescribed by the Animal Ethics Committee of China Pharmaceutical University.

2.13. Type 2 diabetic mice

After acclimatization, the mice were randomly divided into two groups. Mice in the normal control group (n = 8) had free access to ordinary mouse chow whereas those in the other group (n = 45) were fed a high sucrose-high fat diet consisting of 10% lard, 12.5% sucrose, 5% yolk powder, 1% cholesterol, 0.1% cholate, 71.4% basic diet (w/w, the Laboratory Animal Center of Nanjing Qinglongshan, China). After 10 weeks, the mice were fasted for 6 h and intraperitoneally injected with 45 mg/kg STZ (in 0.1 M citrate buffer, pH 4.5) once a day for 3 consecutive days to induce T2D, whereas the normal control mice were given a 0.1 M citrate buffer in equal volume.

After 1 day, the mice were fasted for 6 h and treated with glucose (2 g/kg, p.o.). The blood glucose levels at 0 and 2 h after the glucose treatment were determined. Mice with blood glucose levels of 10.0 mM at 0 min or 13.0 mM at 120 min were considered to have T2D^{16,17}.

2.14. Experimental groups and drug administration

T2D mice were randomly divided into four groups (n = 8) via fasting glucose and were continually maintained on tap water and high-fat diet. The normal control mice had free access to ordinary chow. The mice in the normal group and model group were orally administrated 0.2% carboxymethyl cellulose sodium as a vehicle. The mice in the drug group were orally administered 60 or 120 mg/kg HLYTBE or 200 mg/kg metformin. The administration continued for 10 weeks. The weight and food intake of mice were recorded every 2 days.

2.15. Oral glucose tolerance test (OGTT) and pyruvate tolerance test (PTT)

The OGTT was performed on the ninth week and PTT was performed on the tenth week. The mice were fasted for 6 h and blood samples were collected from the postorbital venous plexus veins (0 min). Immediately after blood collection, all the mice were orally administered glucose at 2.0 g/kg or were intraperitoneally injected with sodium pyruvate at 2.0 g/kg. Blood samples were successively collected at 30, 60, and 120 min after the administration of glucose or pyruvate. The glucose levels in the serum were determined using commercial assay kits according to the manufacturer's directions, and the glucose area under the curve (AUC) was calculated.

2.16. Biochemical assay

In the tenth week after administration, the mice were anaesthetised and blood samples were collected. The blood samples were centrifuged at $2500 \times g$ for 15 min and sera were collected. The levels of blood glucose, GSP, TC, triglyceride, SOD, and GSH-Px in the serum were determined colourimetrically using commercial assay kits according to the directions of the manufacturer. The concentrations of insulin, NEFA, and 8-iso-PGF2 α in the serum were measured using commercial ELISA kits.

2.17. Histopathological analysis

The thoracic aorta was dissected, washed with saline, and fixed by immersing in formalin solution. The aortic arch was dehydrated in an increasing ethanol gradient and embedded in paraffin. The thickness of the aortic arch was about 4 μm . The nucleus was stained with haematoxylin and cytoplasm was stained with eosin. After dehydration and sealing, the cells were examined under an Olympus BX53 microscope.

2.18. Detection of apoptosis of aorta tissue

The apoptosis of aorta tissue was detected by terminal deoxynucleotidyl-transferase-mediated dUTP nick-end labelling (TUNEL) technique. TUNEL staining was performed with formalin-fixed and paraffin-embedded sections at 4- μm thickness using the In Situ Cell Death Detection Kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacture's instructions. The apoptosis of aortic endothelial cells was detected under a light microscope.

2.19. Statistical analysis

All the experimental data were expressed as mean $\pm$ SEM. One-way analysis of variance (ANOVA) followed by Tukey's test or two-tailed Student's *t* test was used to compare the differences between the groups. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. UPLC analysis of flavonoid glycosides, caffeine, and 10 major catechins in HLYTBE

Both HLYT and Lu'an GuaPian tea are special local products in Lu'an city. Thus, we quantified the nine flavonoid glycosides, reported to be present in the Lu'an GuaPian tea, in HLYTBE ¹². Based on the comparison of the retention times of the peaks obtained with those of the reference peaks, we determined the content of the nine flavonoid glycosides in HLYTBE (Supplementary Table 1). The major flavonoid glycosides detected were quercetin-O-Glc-Rha-Glc (9.02 mg/g), kaempferol 3-O-Glc-Rha-Glc (23.30 mg/g), kaempferol 3-O-Glc-Rha (5.64 mg/g), quercetin 3-O-*p*-Cou-Glc-Ara-Rha-Glc (2.31 mg/g), and kaempferol 3-O-*p*-Cou-Glc-Ara-Rha-Glc (1.17 mg/g). Our results suggest that flavonoid glycosides can be easily concentrated through n-butanol extraction.

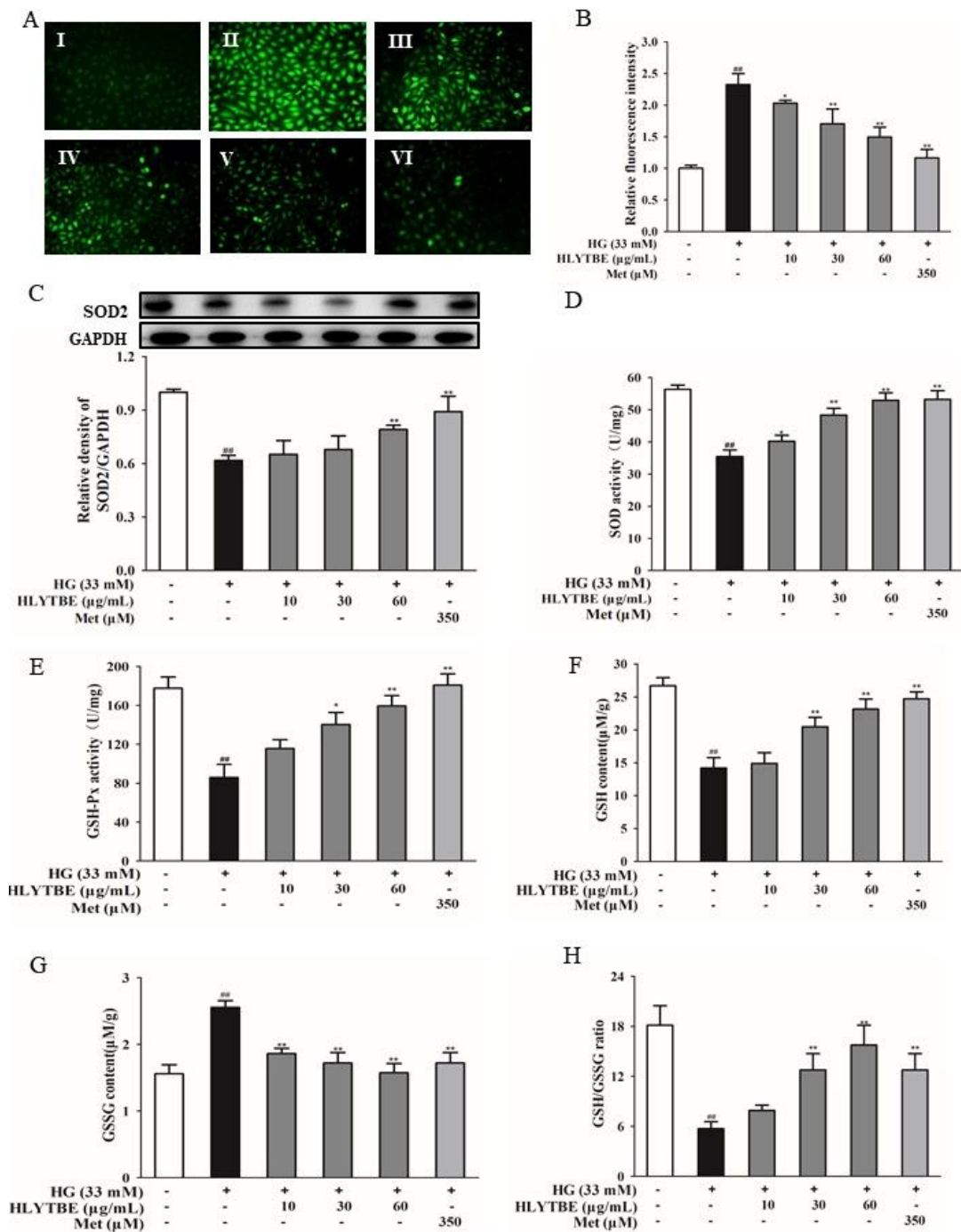
To quantify the characteristic compounds present in tea, caffeine and 10 major catechins were analysed. Supplementary Table 2 presents the content of the 11 compounds in HLYTBE. Comparing the retention times obtained for the samples with those of the reference peaks, and performing quantitative analysis, we determined the

content of gallic catechin (9.32 mg/g), caffeine (43.06 mg/g), epigallocatechin (35.76 mg/g), catechin (11.58 mg/g), epicatechin (12.38 mg/g), epigallocatechin gallate (EGCG, 20.80 mg/g), gallic catechin gallate (3.86 mg/g), epigallocatechin gallate 3''-O-methyl (4.29 mg/g), epicatechin gallate (11.72 mg/g), catechin gallate (1.53 mg/g), epicatechin gallate 3''-O-methyl (0.31 mg/g) in HLYTBE.

3.2. Effect of HLYTBE on oxidative stress in HUVECs induced by HG

ROS production in endothelial cells was observed using the specific fluorescent dye, DCFH-DA. The ROS production in HG-treated HUVECs was significantly increased, when compared to that in the untreated group. Treatment with HLYTBE remarkably suppressed the ROS production by 21.7%, 46.5%, and 62.4% at 10, 30, and 60 µg/mL, respectively (Fig. 1A, B). SOD can catalyse the disproportionation of superoxide anion radicals to produce oxygen and hydrogen peroxide and remove excess free radicals. The protein expression of SOD2 in HLYTBE 60 µg/mL group was remarkably increased (Fig. 1C). The SOD and GSH-Px activities in HG-treated HUVECs are significantly decreased by 0.63- and 0.48-fold, respectively, when compared to that in the untreated group. HLYTBE (30 and 60 µg/mL) treatment could remarkably increase the SOD and GSH-Px activity with 1.36- and 1.49-fold, and 1.64 and 1.86-fold of the respective activities in the HG only treated group (Fig. 1D, E). In addition, the effects of HLYTBE on the changes in the GSH/GSSG content were also determined in HG-treated HUVECs (Fig. 1F, G, H). The results showed that the GSH content and the ratio of GSH/GSSG were decreased, whereas the GSSG content was increased in the presence of HG. However, treatment of HUVECs with HLYTBE

could attenuate the decrease in the GSH content and increase in the GSSG level, thereby, elevating the GSH/GSSG ratio. These results suggested that HLYTBE could inhibit oxidative stress in HUVECs induced by HG.



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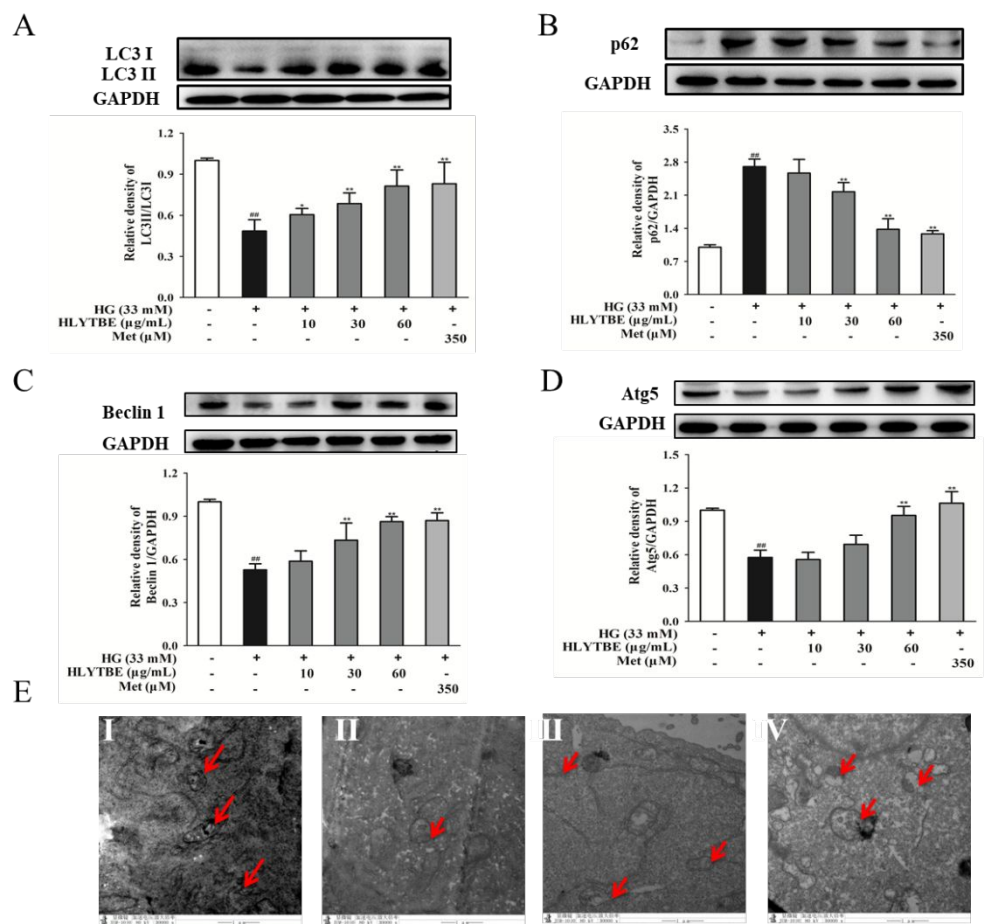
311 **Fig. 1** Effect of HLYTBE on oxidative stress in HG-treated HUVECs. The cells were

312 treated with HLYTBE (10, 30, 60 $\mu\text{g/mL}$) or Metformin (Met, 350 μM) in the
313 presence or absence of glucose (33 mM) for 48 h. (A) The ROS levels were measured
314 by fluorescence of DCFH-DA using a Olympus fluorescence microscope (200 $\times$). (I)
315 untreated group; (II) HG only treated group; (III) HLYTBE 10 $\mu\text{g/mL}$; (IV) HLYTBE
316 30 $\mu\text{g/mL}$; (V) HLYTBE 60 $\mu\text{g/mL}$; (VI) Met 350 μM . (B) The fluorescence intensity
317 was quantified by densitometry using the Image J software. (C) Expression of SOD2
318 protein was determined by western blot analysis. Total SOD (D) activity, GSH-Px (E)
319 activity, and the level of GSH (F) and GSSG (G) were determined using a microplate
320 reader. The ratio of GSH/GSSG (H) was measured. The results are presented as
321 means $\pm$ SEM ($n = 3\sim 4$). $^{###}P < 0.01$ vs. untreated group, $^{*}P < 0.05$, $^{**}P < 0.01$ vs. HG
322 only treated group.

323 3.3. Effect of HLYTBE on autophagy in HUVECs induced by HG

324 The effect of HLYTBE on autophagy-related protein expression in HG-treated
325 HUVECs was evaluated. The LC3II/LC3I ratio (Fig. 2A) and the protein expression
326 of Beclin1 (Fig. 2B) and Atg5 (Fig. 2C) were significantly decreased, whereas the
327 expression of p62 (Sequestosome-1) (Fig. 2D) was remarkably increased in HUVECs
328 exposed to HG. HLYTBE (10, 30, 60 $\mu\text{g/mL}$) treatment could significantly increase
329 the LC3II/LC3I ratio with 1.16-, 1.35-, and 1.52-fold of the ratio in the HG only
330 treated group, respectively. At the same time, HLYTBE (30 and 60 $\mu\text{g/mL}$) treatment
331 could significantly reduce the expression of p62 and the inhibition was about 30.3%
332 and 77.1%, respectively. In addition, HLYTBE (30 and 60 $\mu\text{g/mL}$) treatment could
333 significantly up-regulate the expression of Beclin1, whereas HLYTBE 60 $\mu\text{g/mL}$

334 treatment could remarkably increase the expression of Atg5. These results
335 demonstrated that HLYTBE could enhance the autophagy related protein expression
336 in HUVECs treated with HG. Furthermore, TEM was performed to visualize the
337 autophagosomes in HUVECs. Consistent results showing fewer autophagosomes in
338 the HG only treated group than that in the untreated group were obtained. On the
339 contrary, there were more autophagosomes in the HLYTBE group (Fig. 2E). These
340 results showed that HLYTBE could improve the autophagy in HG-treated HUVECs.



341

342 **Fig. 2** Effect of HLYTBE on autophagy in HG-treated HUVECs. The cells were
343 treated with HLYTBE (10, 30, 60 μg/mL) or Met (350 μM) in the presence or absence
344 of glucose (33 mM) for 48 h. The protein expression levels of LC3 (A), p62 (B),
345 Beclin1 (C), and Atg5 (D) were determined by western blot analysis. (E)

Autophagosomes in HUVECs were observed by TEM (30000 $\times$). Red arrows represent autophagosomes. (I) untreated group; (II) HG only treated group; (III) HLYTBE 60 μ g/mL; (IV) Met 350 μ M. The results are presented as means $\pm$ SEM (n = 3~4). ^{##} $P < 0.01$ vs. untreated group, ^{*} $P < 0.05$, ^{**} $P < 0.01$ vs. HG only treated group.

3.4. Effect of HLYTBE on AMPK/mTOR signalling pathway in HG-treated HUVECs

The AMPK/mTOR pathway plays an important role in regulating autophagy and serves as an upstream signal for autophagy. As shown in Fig. 3A, HLYTBE (10, 30, 60 μ g/mL) treatment could significantly increase the p-AMPK levels by 1.25-, 1.63-, and 1.81-fold of the levels in the HG only treated group, respectively. The phosphorylation of mTOR was dramatically increased compared with that in the untreated group. As expected, HLYTBE (30 and 60 μ g/mL) significantly suppressed the phosphorylation of mTOR by 33.7% and 77.5%, respectively (Fig. 3B). Furthermore, cells co-treated with HLYTBE and Compound C (an AMPK inhibitor) showed a significant increase in the expression of p-mTOR (Fig. 3C) and p62 (Fig. 3D) compared with that in the cells treated with HLYTBE. These results suggested that HLYTBE significantly up-regulated the AMPK/mTOR signalling pathway and enhanced the autophagy via the AMPK-dependent pathway in vascular endothelial cells evoked by HG.

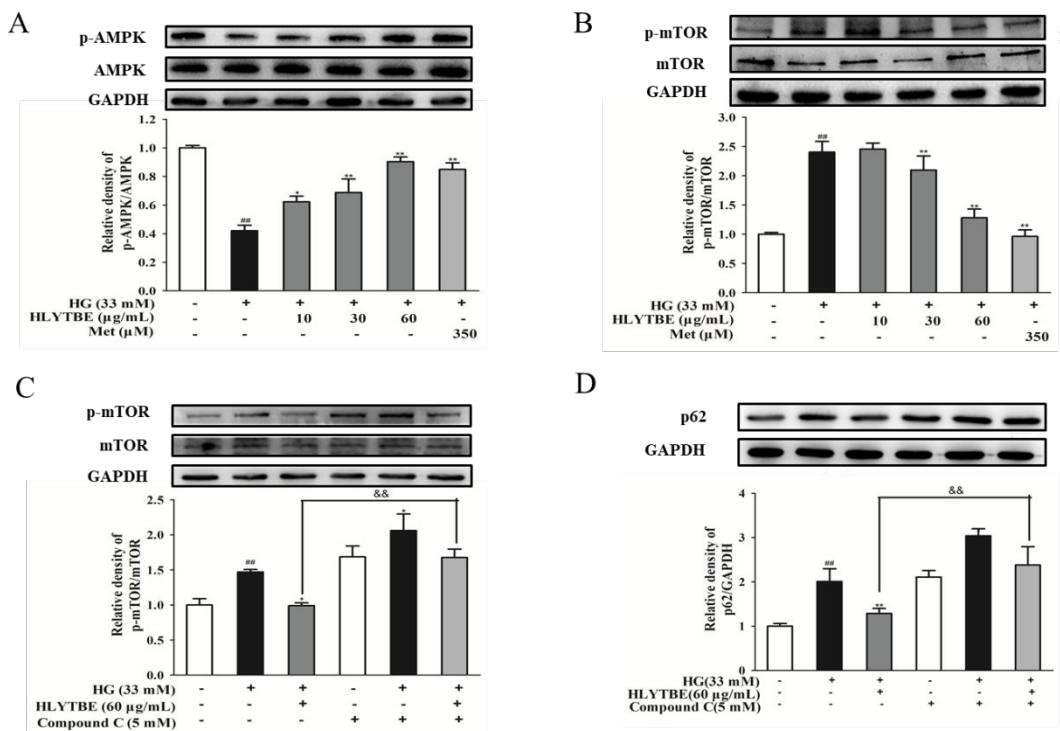


Fig. 3 Effect of HLYTBE on the AMPK/mTOR signalling pathway in HG-treated HUVECs. The cells were treated with HLYTBE (10, 30, 60 μg/mL) or Met (350 μM) in the presence or absence of glucose (33 mM) for 48 h. The levels of p-AMPK (A) and p-mTOR (B) were determined by western blot analysis. The cells were treated with HLYTBE (60 μg/mL) or an AMPK inhibitor, Compound C (5 mM) in the presence or absence of glucose (33 mM) for 48 h. The expression levels of p-mTOR (C) and p62 (D) were determined by western blot analysis. The results are presented as means ± SEM (n = 3~4). ^{##} *P* < 0.01 untreated group, ^{*} *P* < 0.05, ^{**} *P* < 0.01 vs. HG only treated group; ^{&&} *P* < 0.01 vs. HLYTBE (60 μg/mL) group.

3.5. Protective effects of HLYTBE on apoptosis of HUVECs induced by HG

To determine whether HLYTBE reduced the apoptosis in HG-treated HUVECs, we performed Hoechst 33342 staining. As illustrated in Fig. 4A, following HG treatment, HUVECs exhibited apoptotic characteristics—condensation or fragmentation of

chromatin—which were evident by emission of strong fluorescence. The apoptotic rate of cells in the HG only treated group was significantly higher than of those in the untreated group (Fig. 4B). When the cells were treated with HLYTBE, the apoptotic rate was reduced, and the inhibition was about 29.7%, 44.9%, and 87.0 % at 10, 30, and 60 $\mu\text{g/mL}$, respectively. The release of Cyt.c from mitochondria into the cytoplasm is a key initial step in apoptosis. We found that treatment with HLYTBE (10, 30, 60 $\mu\text{g/mL}$) could increase mitochondrial Cyt.c protein expression and decrease cytoplasmic protein expression in a dose-dependent manner in HUVECs treated with HG (Fig. 4C, D). Cleaved caspase-3 is involved in the execution of apoptosis and regulates the entire process. Consistently, treatment with HLYTBE (10, 30, 60 $\mu\text{g/mL}$) could remarkably decrease the expression of cleaved caspase-3 in a dose-dependent manner and the inhibition was about 32.9%, 67.4%, and 94.8%, respectively (Fig. 4E). The results suggested that HLYTBE could inhibit mitochondria-mediated apoptosis in HG-treated vascular endothelial cells.

To further demonstrate that the anti-apoptotic effect of HLYTBE contributed to the promotion of autophagy, we examined its effect on the expression of Cyt.c and cleaved caspase-3, by incubating HUVECs with HG and an autophagy inhibitor, 3-methyladenine (3-MA). The results showed that cells co-treated with HLYTBE and 3-MA significantly increased the expression of Cyt.c (Fig. 4F) and partially increased the expression of cleaved caspase-3 (Fig. 4G) compared with that in the cells treated with HLYTBE. Taken together, these results suggested that HLYTBE protected endothelial cells from HG-induced apoptosis by increasing the autophagy level.

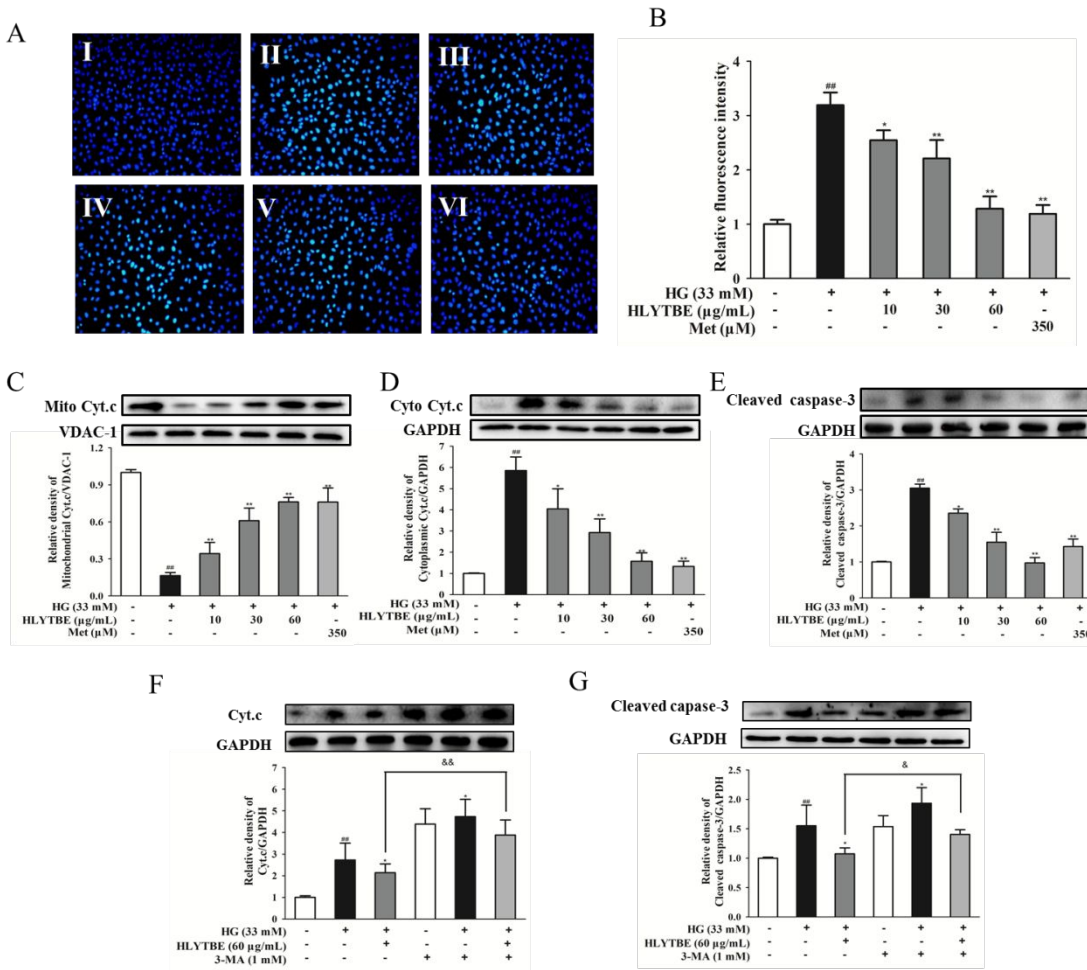


Fig. 4 Effect of HLYTBE on apoptosis in HG-treated HUVECs. The cells were treated with HLYTBE (10, 30, 60 $\mu\text{g/mL}$) or Met (350 μM) in the presence or absence of glucose (33 mM) for 48 h. (A) Cell apoptosis was detected by Hoechst staining using a Olympus fluorescence microscope (400 $\times$). (I) untreated group; (II) HG only treated group; (III) HLYTBE 10 $\mu\text{g/mL}$; (IV) HLYTBE 30 $\mu\text{g/mL}$; (V) HLYTBE 60 $\mu\text{g/mL}$; (VI) Met 350 μM . (B) The fluorescence intensity was quantified by densitometry using the Image J software. The expression levels of Cyt.c in mitochondria (C) and cytoplasm (D) and cleaved caspase-3 (E) were investigated by western blot analysis. The cells were treated with HLYTBE (60 $\mu\text{g/mL}$) or with 3-MA (1 mM), in the presence or absence of glucose (33 mM) for 48

h. The expression levels of Cyt.c (F) and cleaved caspase-3 (G) were determined by western blot analysis. The results are presented as means $\pm$ SEM ($n = 3$). $^{###}P < 0.01$ vs. untreated group, $^{**}P < 0.01$ vs. HG only treated group. $^{\&}P < 0.05$ vs. HLYTBE (60 $\mu\text{g/mL}$) group.

3.6. Effect of HLYTBE on OGTT, PTT, and IR index in diabetic mice

The glucose tolerance and pyruvate tolerance in the diabetic control group were significantly impaired compared with those in the normal group. Compared with the diabetic control group, HLYTBE (60 and 120 mg/kg) groups significantly improved the glucose and pyruvate tolerance, and the inhibition ratios of AUC were 30.1% and 28.3% (Fig. 5A), and 49.7% and 52.0%, respectively (Fig. 5B). Furthermore, HLYTBE (60 and 120 mg/kg) treatment significantly decreased the IR index compared with that in the diabetic control group, and the inhibition was 76.5% and 65.8%, respectively (Fig. 5C). These results suggested that HLYTBE could remarkably ameliorate IR in diabetic mice.

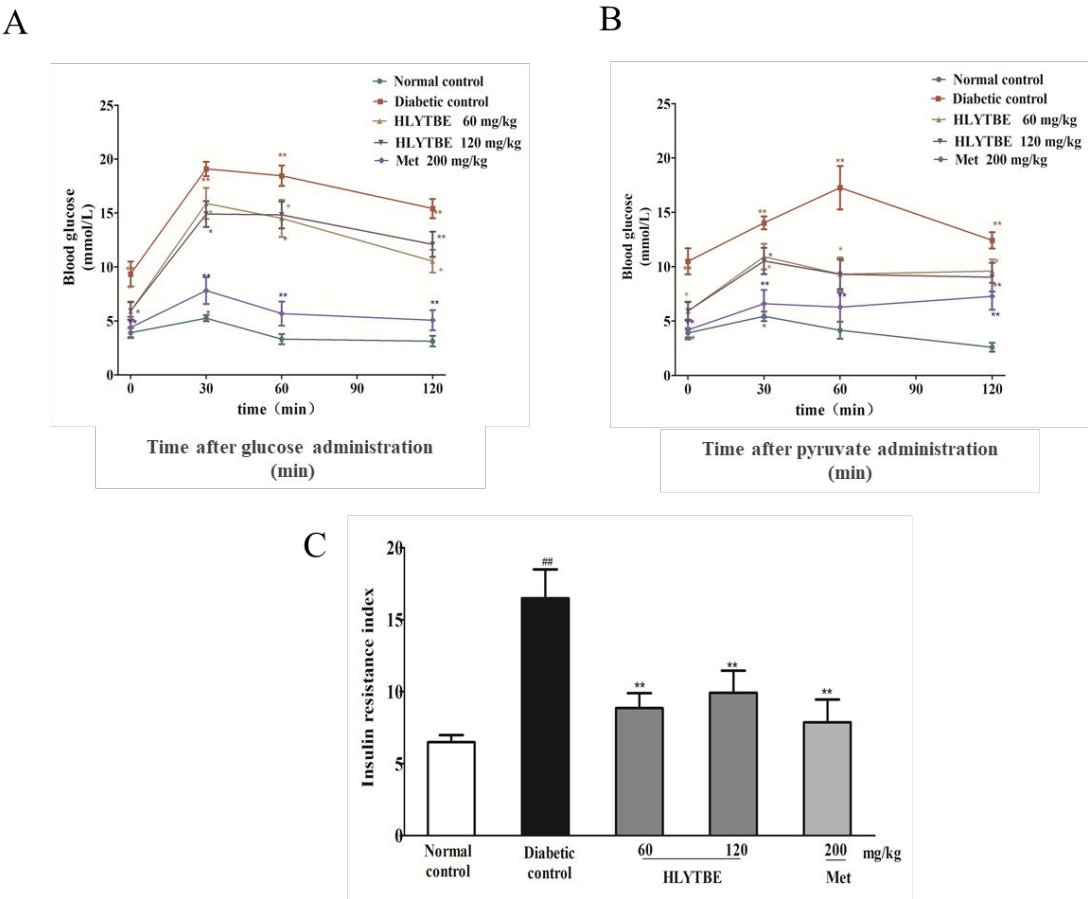


Fig. 5 Effects of HLYTBE on OGTT, PTT, and IRI in diabetic mice. Diabetic mice were administrated HLYTBE (60 and 120 mg/kg) or Met (200 mg/kg) for 10 weeks (n = 6–8). (A) OGTT. (B) PTT. (C) IRI = fasting blood glucose $\times$ fasting serum insulin/22.5. $^{###}P < 0.01$ vs. Normal control group, $^{*}P < 0.05$, $^{**}P < 0.01$, vs. Diabetic control group.

3.7. Effect of HLYTBE on glycolipid metabolism and oxidative stress in diabetic mice

To assess the effect of HLYTBE on the glucose metabolism in diabetic mice, we evaluated the levels of serum FBG and GSP in each group. As shown in table 1, compared with the diabetic control group, HLYTBE (60 and 120 mg/kg) significantly decreased the serum FBG and GSP levels by 66.1% and 80.6%, and 59.7% and 72.7%,

439 respectively. Additionally, diabetic mice showed an abnormal lipid metabolism.
440 Compared with the diabetic control group, HLYTBE (60 and 120 mg/kg) significantly
441 decreased the levels of TC, triglycerides, and NEFA, and their inhibition ratios were
442 46.1% and 41.3%, 50.2% and 51.7%, and 74.9% and 86.7%, respectively. Oxidative
443 stress is involved in the development and progression of diabetic vascular disease ¹⁸.
444 HLYTBE (60 and 120 mg/kg) could increase the SOD activity by 1.89- and 1.94-fold
445 compared with that in the diabetic control group, respectively. Moreover, HLYTBE
446 (60 and 120 mg/kg) could increase the GSH-Px activity by 1.43- and 1.58-fold
447 compared with that in the diabetic control group, respectively. Furthermore, compared
448 with the diabetic control group, HLYTBE (60 and 120 mg/kg) significantly decreased
449 the levels of 8-iso-PGF2 α in the serum by 93.4% and 75.7%, respectively. These
450 results suggested that HLYTBE could regulate the abnormal lipid metabolism and
451 exhibit potent antioxidant activity to reduce oxidative damage.

453 **TABLE 1** Effect of HLYTBE on glycolipid metabolism and oxidative stress in
454 diabetic mice

Parameter	Normal	Diabetic	HLYTBE	HLYTBE	Met
	control	control	60 mg/kg	120 mg/kg	200 mg/kg
FBG (mM)	6.93±1.65	12.98±3.94 ^{##}	8.70±2.76 ^{**}	7.74±2.13 ^{**}	6.78±2.90 [*]
GSP (mM)	4.46±0.43	5.24±0.23 ^{##}	4.78±0.51 ^{**}	4.68±0.53 ^{**}	4.42±0.58 [*]
TG (mM)	2.08±0.43	2.83±0.16 ^{##}	2.45±0.40 [*]	2.44±0.28 [*]	2.03±0.89 [*]
TC (mM)	1.80±0.31	5.02±0.73 ^{##}	3.53±0.79 ^{**}	3.69±0.82 ^{**}	3.26±0.66 ^{**}
NEFA (μM)	305.39±99.87	697.47±269.30 ^{##}	403.24±260.11 [*]	357.25±107.02 ^{**}	359.19±99.95 ^{**}
SOD(U/mL)	219.84±50.79	111.36±56.89 ^{##}	209.96±26.34 ^{**}	215.73±67.75 ^{**}	225.01±42.42 ^{**}
GSH-Px(U/ml)	235.34±26.39	141.00±33.47 ^{##}	202.68±25.00 ^{**}	223.87±17.79 ^{**}	185.15±26.19 ^{**}
8-iso-PGF2α(pg/mL)	133.61±20.44	175.52±23.46 ^{##}	143.80±21.17 [*]	136.37±25.71 ^{**}	146.39±25.59 [*]

455 Diabetic mice were administrated HLYTBE (60 and 120 mg/kg) or Met (200 mg/kg)
456 for 10 weeks (n = 6 - 8). The levels of fasting blood glucose, GSP, TC, TG, NEFA,
457 total SOD, GSH-Px and 8-iso prostaglandin F2 in the serum were measured. ^{##}*P* <
458 0.01 vs. Normal control group, ^{*}*P* < 0.05, ^{**}*P* < 0.01, vs. Diabetic control group.

459 **3.8. Effect of HLYTBE on the apoptosis and histopathological damage of**
460 **thoracic aorta arch in diabetic mice**

461 As shown in Fig. 6A, the number of TUNEL-positive nuclei in aortic sections of the
462 diabetic control group was remarkably increased compared with that in the normal
463 control group. Treatment with HLYTBE or Met significantly decreased the number of
464 TUNEL-positive nuclei compared with that in the diabetic control group. These

results suggest that HLYTBE could alleviate the apoptosis of cells in the aorta of diabetic mice. We next assessed the effect of HLYTBE on the histopathological changes in the thoracic aorta arch in diabetic mice. As shown in Fig. 6B, the intima of thoracic aortic arch in normal mice was smooth and clear. In diabetic control group, the intima of thoracic artery arch was damaged, the intima was thickened, the texture was not clear, and there was fat deposition under the intima, and the cell swelling was obvious. These results suggested that HLYTBE could significantly attenuate the aortic arch vascular lesions in type 2 diabetic mice.

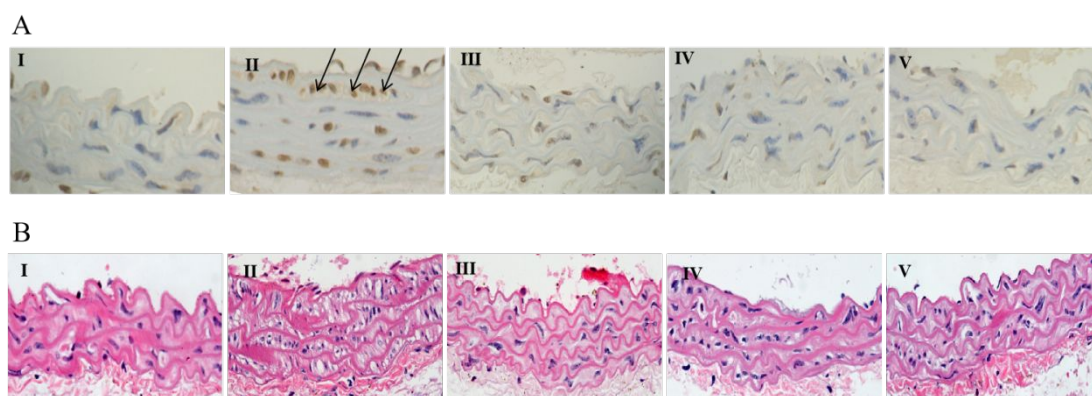


Fig. 6 Effect of HLYTBE on the apoptosis, and histopathological changes in the thoracic aorta arch in diabetic mice. Diabetic mice were administrated HLYTBE (60 and 120 mg/kg) or Met (200 mg/kg) for 10 weeks. (A) The samples were sectioned and processed using the TUNEL Staining Kit and DAB. The apoptotic nuclei stained with DAB were brownish-yellow (Black arrow). (B) The histopathological changes in the thoracic aorta were observed by optical microscopy: HE (400 $\times$). (I) Normal control group; (II) Diabetic control group; (III) HLYTBE 60 mg/kg group; (IV) HLYTBE 120 mg/kg group; (V) Met 200 mg/kg group.

4. Discussion

Macro-vascular diabetic complications mainly represented by atherosclerotic disease are principally caused by prolonged exposure to hyperglycaemic conditions. A high glucose environment results in vascular endothelial dysfunction, mainly related to oxidative stress and apoptotic cascade, but the details are still unclear¹⁹.

Oxidative stress plays a key role in vascular endothelial injury and is considered as an early mechanism in many cardiovascular diseases^{20,21}. Studies have shown that hyperglycaemia-induced ROS production plays a critical role in the apoptosis of endothelial cells²². ROS can increase the level of hydrogen peroxide. GSH-Px pdetoxifies hydrogen peroxide by converting two GSH molecules to the oxidized form, GSSG, which in turn is reduced back to GSH by glutathione reductase to constitute a redox cycle²³. GSH is an important antioxidant and free radical scavenger *in vivo*. In addition, GSH-Px can scavenge ROS to protect the structure and function of cell membrane²⁴. It has been shown in previous studies that yellow tea leaves are rich in phenolic compounds with antioxidant activity²⁵. Consistently, in this study, we demonstrate that HLYTBE could reduce HG-induced oxidative stress in vascular endothelial cells by reducing ROS production and GSSG content, enhancing the expression of SOD2 and SOD activity, increasing the GSH-Px activity, and elevating the GSH level and GSH/GSSG ratio (Fig. 1). These results indicate that HLYTBE could be helpful in treating endothelial oxidative stress associated vascular endothelial injury.

Autophagy plays an important role in vascular complications of diabetes, and it is closely related to oxidative stress and apoptosis²⁶. Aging or damaged organelles are

absorbed by autophages and lysosomes are degraded into macromolecules in response to nutritional deficiency or other stress-related signals. In the process of autophagy, Atg5 is involved in the formation of the Atg5–Atg12 complex, which is essential for the generation of autophagosomes²⁷. Beclin1 is also a molecule that is essential for the formation of autophagosomes. As a multifunctional ubiquitin-binding protein, p62 is involved in the degradation of proteins in the ubiquitin–proteasome and autophagy–lysosomal system. Studies have shown that HG can directly inhibit the autophagic flux of vascular endothelial cells²⁸. We found that LC3-II/LC3-I, Atg5, and Beclin1 levels were weakened and p62 expression was increased by HG treatment. Moreover, we observed that HLYTBE enhanced the LC3-II/LC3-I ratio, the expression of Atg5 and Beclin1, and decreased the expression of p62 in HG-treated HUVECs (Fig. 2). Next, by TEM analysis, we found that treatment with HLYTBE (60 µg/mL) significantly increased the number of autophagosomes in HUVECs. These results suggest that HLYTBE can up-regulate autophagy in HUVECs induced by HG and HLYTBE may be an autophagy agonist.

The AMPK/mTOR pathway is important for regulating autophagy and serves as an upstream target of autophagy^{29, 30}. AMPK is stimulated by changes in cellular energy and responds to energy stress. AMPK can inhibit the activity of the mTOR complex (mTORC1) by directly phosphorylating Raptor³¹. Several studies have shown that AMPK is a positive regulator of autophagy and inhibits the mTOR pathway^{32, 33}. In this study, we observed that HLYTBE could remarkably up-regulate the phosphorylation of AMPK and down-regulated that of mTOR in vascular

endothelial cells evoked by HG (Fig. 3). To confirm that the effect of HLYTBE in the enhancing autophagy was mediated by the AMPK/mTOR signalling pathway, we treated vascular endothelial cells with Compound C. This treatment restored the expression of p-mTOR and p62 induced by HG, and the expression of these proteins was remarkably reduced in the Compound C plus HLYTBE group. These results suggested that the enhancement of autophagy by HLYTBE was through the up-regulation of the AMPK/mTOR signalling pathway.

The apoptosis of endothelial cells plays an important role in the development and pathogenesis of many vascular diseases, including atherosclerosis and vascular remodelling. The caspase family forms the core of the programmed death signal network, among which caspase-3 is the main executor of apoptosis and plays an important role in apoptosis. Additionally, studies have shown that a large amount of Cyt.c is released from mitochondria during the process of apoptosis, and accelerates it. We show that HLYTBE could reduce the expression of cleaved caspase-3 and Cyt.c and inhibit the apoptosis in HUVECs induced by HG. For cells with an impaired function, it is impossible to achieve a balance between autophagy and apoptosis, and they ultimately undergo apoptosis³⁴. The autophagy of endothelial cells can resist apoptosis under certain stress conditions³⁵. In the presence of the autophagy inhibitor, 3-MA, autophagy was significantly inhibited and the levels of cleaved caspase-3 and Cyt.c were significantly increased. Moreover, the inhibitory effects of HLYTBE on the expression of Cyt.c and cleaved caspase-3 were reversed (Fig. 4). These data suggested that HLYTBE could reduce apoptosis in endothelial cells under HG

conditions by promoting autophagy.

The main characteristics of T2DM are impaired insulin secretion and peripheral IR, which can lead to disorders in glucose and lipid metabolism³⁶. There are several reports on the hypoglycaemic and lipid-lowering effects of flavonoids and catechins that are ubiquitously present in tea³⁷⁻⁴⁰. Green, black, and Pu'er tea have pharmacological effects related to the regulation of lipid metabolism, as well as anti-inflammatory and anti-obesity effects⁴¹. Studies have shown that green tea polyphenols alleviate the inhibition of autophagy induced by HG in endothelial cells⁴². Yellow tea has significant hypoglycaemic effect and can alleviate the disorder of glucose metabolism in ICR mice induced by a high-fat diet⁴. In the study by Han, the effects of tea water infusion could be due to the presence of polar ingredients in tea. Accordingly, we used HLYTBE, a polar fraction (butanol fraction) of Huoshan large-leaf yellow tea, in the present study. We found that the flavonoid glycosides, non-gallated catechins (gallocatechin, epigallocatechin, catechin, epicatechin), and caffeine were significantly enriched, which may contribute to the effects of HLYTBE. The decrease in the most abundant compound, EGCG, may lead to hundred-fold increase in the amount of related 8- or 6- ethylpyrrolidinone substituted EGCG (flavoalkaloids), which may also contribute to the health benefits of large yellow tea³. There is some evidence that EGCG has the capacity to scavenge toxic reactive metabolic wastes⁴³. Yellow tea contains (–)-epicatechin, which has beneficial effects on lipid metabolism and cardiovascular diseases⁴⁴.

Our results show that HLYTBE could significantly improve impaired IPTT and

OGTT as well as IR in diabetic mice induced by high sucrose-high fat diet and STZ (Fig. 5). HLYTBE also reduced fasting blood glucose, GSP, TC, triglyceride, and free fatty acid levels. These results suggested that HLYTBE could regulate blood glucose and lipid metabolism, and reduce IR in diabetic mice. Type 2 diabetes mellitus is associated with increased oxidative stress due to the overproduction of ROS and decreased efficiency of the antioxidant defences. The antioxidant enzymes, such as SOD, GSH-Px, and catalase, contribute to eliminate superoxides, hydrogen peroxide, and hydroxyl radicals. F2-isoprostanes are the end products of free radical attack on cell membrane phospholipids. 8-Iso-PGF2 α is considered to be a reliable biomarker for evaluating oxidative stress *in vivo*⁴⁵. HLYTBE increased the activities of SOD and GSH-Px and decreased the level of 8-iso-PGF2 α in the serum of diabetic mice (Table 1). These results suggested that HLYTBE could suppress oxidative stress and reduce oxidative damage in diabetic mice. Vascular endothelial dysfunction is recognized as the initial step in diabetic vascular complications, which might be caused by hyperglycaemia-induced apoptosis of vascular endothelial cells⁴⁶. HLYTBE could significantly decrease the apoptosis of endothelial cells in the thoracic aorta (Fig. 6A). Moreover, HLYTBE could remarkably improve the structure and morphology of endothelial cells of the thoracic aorta in diabetic mice (Fig. 6B). These results were consistent with the results obtained in vascular endothelial cells *in vitro*.

5. Conclusion

This study demonstrates that HLYTBE could significantly improve autophagy by increasing the number of autophagosomes, up-regulating the expression of LC3II,

Beclin1, and Atg5, inhibiting the expression of p62 and oxidative stress, reducing apoptosis by down-regulation of the expression of Cyt.c and cleaved caspase-3 in HG-treated HUVECs. In addition, HLYTBE protected vascular endothelial cells against hyperglycaemia-induced injury via the AMPK/mTOR signalling pathway. Furthermore, HLYTBE could effectively improve glucose and lipid metabolism and insulin resistance, enhance antioxidant enzyme activity, and alleviate arterial vascular injury in diabetic mice. These results provide a theoretical basis for the prevention or alleviation of diabetic vascular complications by large-leaf yellow tea.

Author contributions

Pu Wang, Feihua Wu and Guanhu Bao conceived and designed the experiments; Pu Wang, Yihai Huang, Jiayue Ren, Yuezhao Rong, Lili Fan, Peng Zhang, Xueying Zhang, Junxiao Xi, Shuying Mao, Min Su and Baobao Zhang performed the experiments; Pu Wang and Yihai Huang analyzed the data; Pu Wang, Yihai Huang, Guanhu Bao and Feihua Wu wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Supplementary Table 1. Content of nine flavonoid glycosides in HLYTBE

Flavonoid glycosides	HLYTBE (mg/g)
vitexin 4''-O-Glc	0.14 ± 0.002
Q 3-O-Glc-Rha-Glc	9.02 ± 0.074
K 3-O-Glc-Rha-Glc	23.30 ± 0.691
K 3-O-Glc-Rha	5.64 ± 0.144
K 3-O-Glc	0.42 ± 0.006
Q 3-O- <i>p</i> -Cou-Glc-Ara-Rha-Glc	2.31 ± 0.059
K 3-O- <i>p</i> -Cou-Glc-Ara-Rha-Glc	1.17 ± 0.054
Q 3-O- <i>p</i> -Cou-Glc-Rha-Glc	0.94 ± 0.010
K 3-O- <i>p</i> -Cou-Glc-Rha-Glc	0.25 ± 0.004

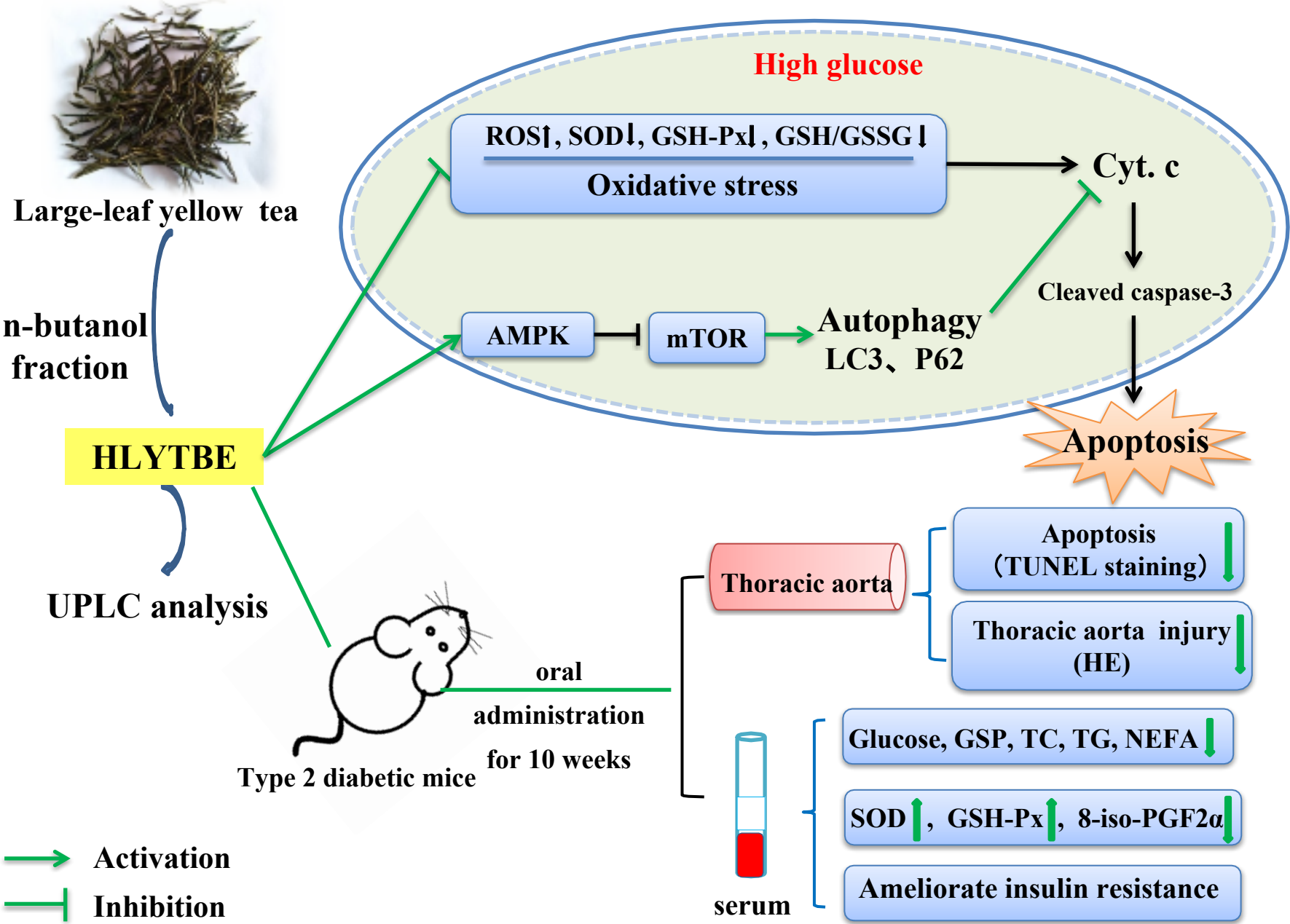
HLYTBE, n-butanol fraction of Huoshan large-leaf yellow tea extract; Q, quercetin;

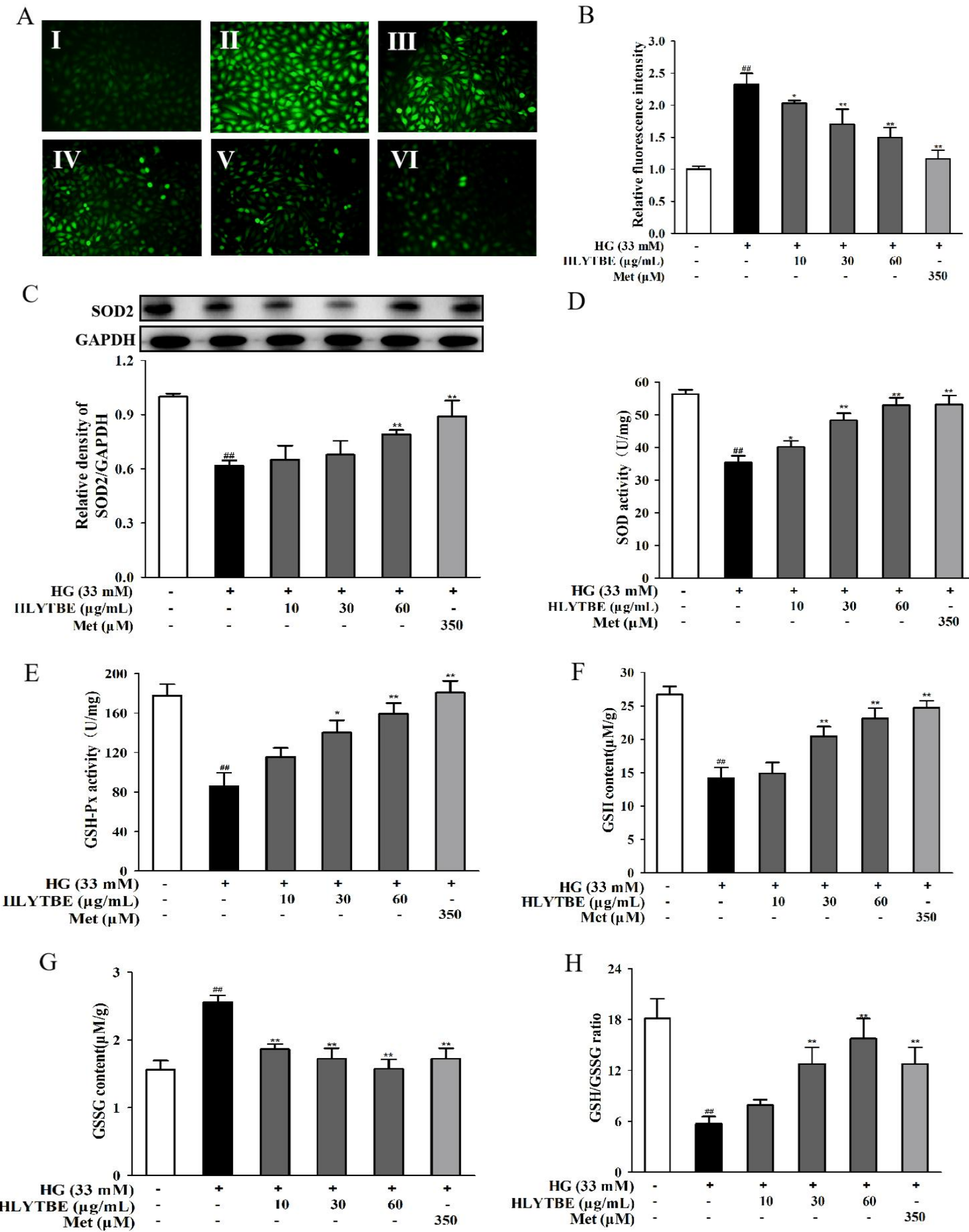
K, kaempferol. Results of three experiments are presented as mean ± SD.

Supplementary Table 2. Content of ten major tea polyphenols and caffeine in HLYTBE

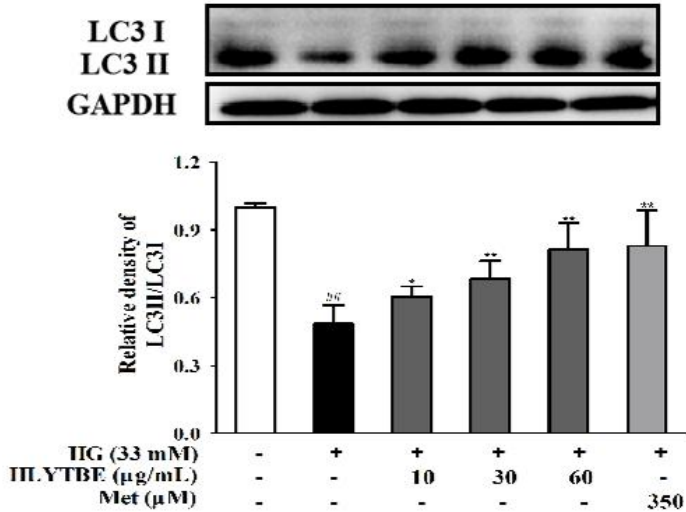
Tea polyphenols	HLYTBE (mg/g)
gallocatechin	9.32 ± 0.934
caffeine	43.06 ± 0.874
epigallocatechin	35.76 ± 0.162
catechin	11.58 ± 0.500
epicatechin	12.38 ± 0.567
epigallocatechin gallate	20.80 ± 0.615
gallocatechin gallate	3.86 ± 0.156
epigallocatechin gallate 3''-O-methyl	4.29 ± 0.266
epicatechin gallate	11.72 ± 0.700
catechin gallate	1.53 ± 0.025
epicatechin gallate 3''-O-methyl	0.31 ± 0.002

HLYTBE, n-butanol fraction of Huoshan large-leaf yellow tea extract. Results of three experiments are presented as mean $\pm$ SD.

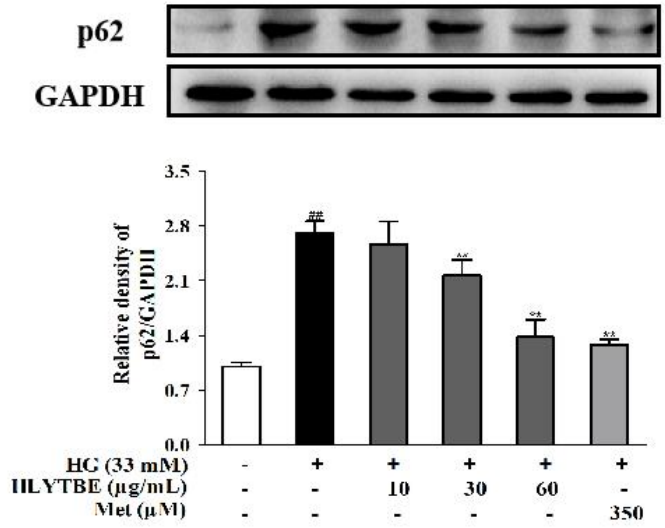




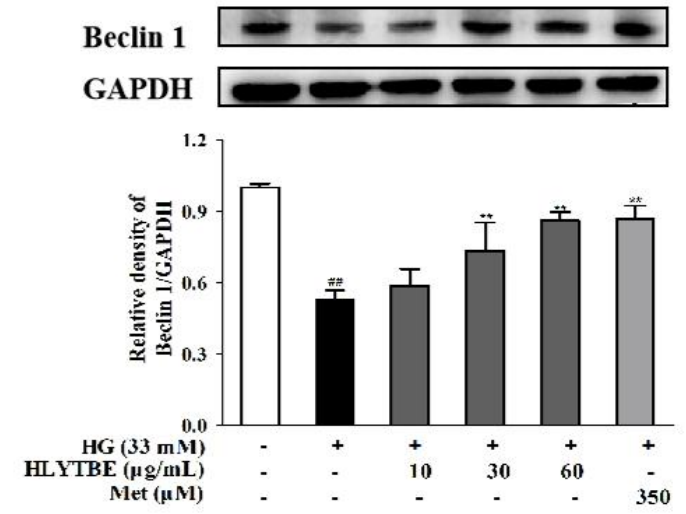
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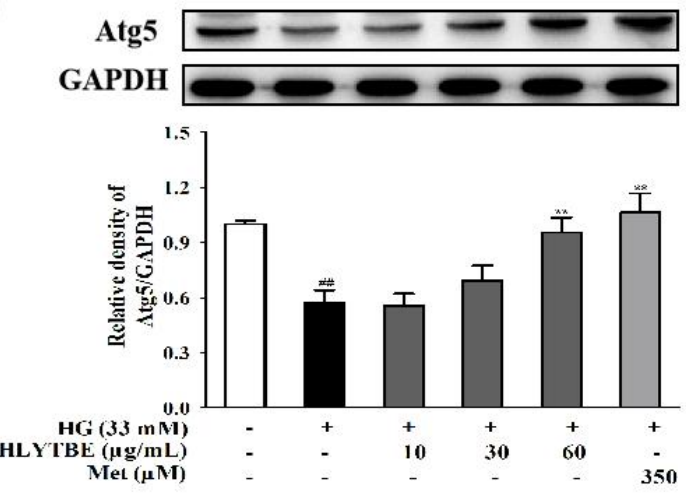
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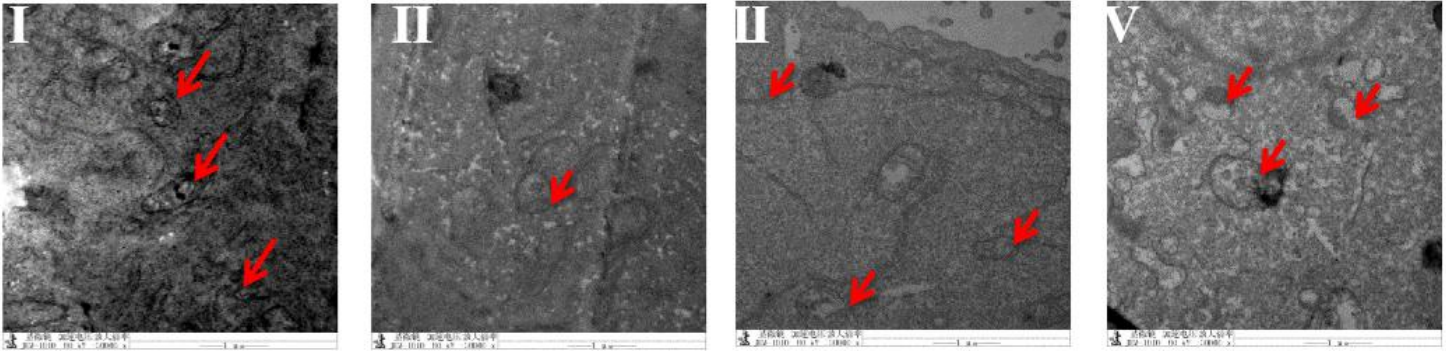
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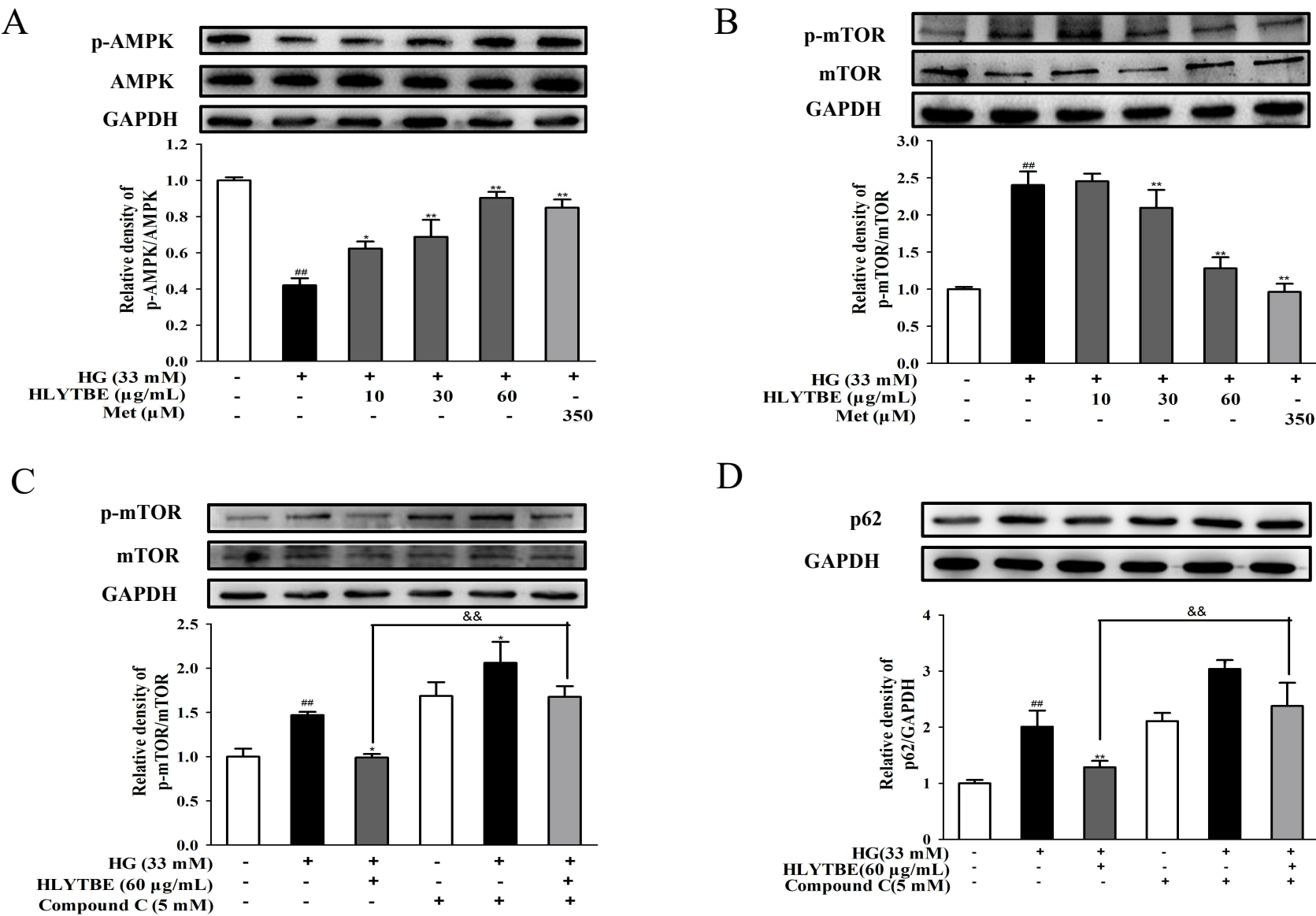


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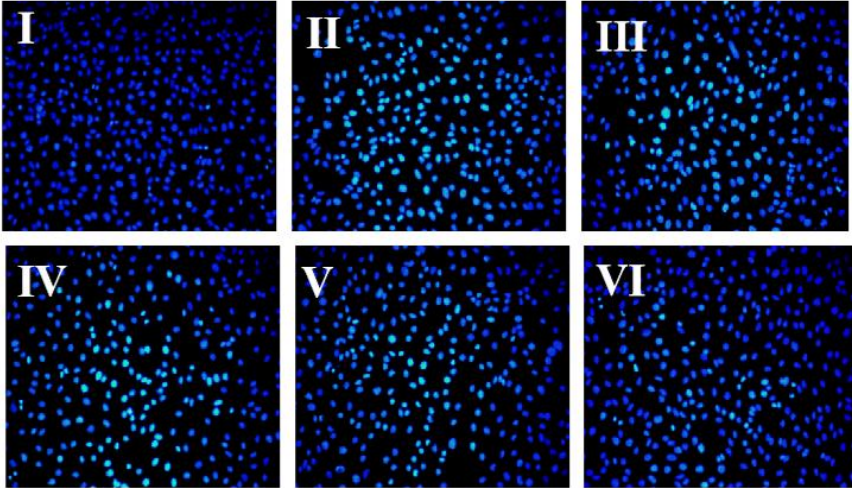


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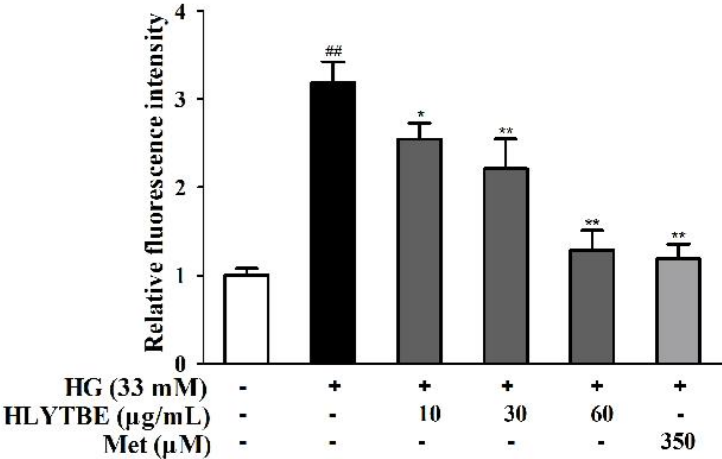




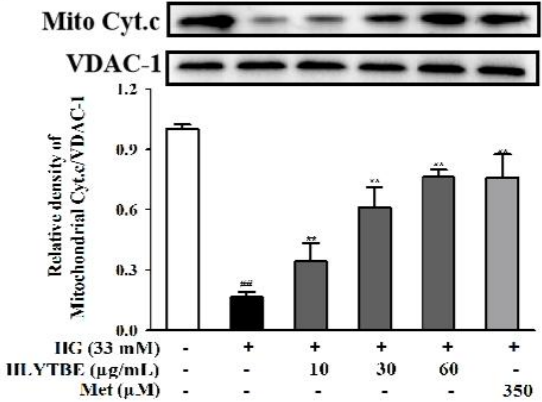
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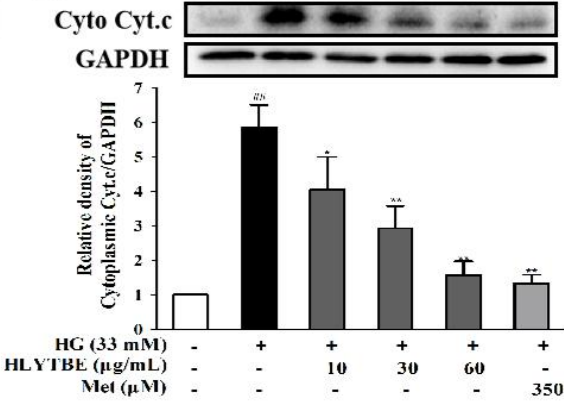
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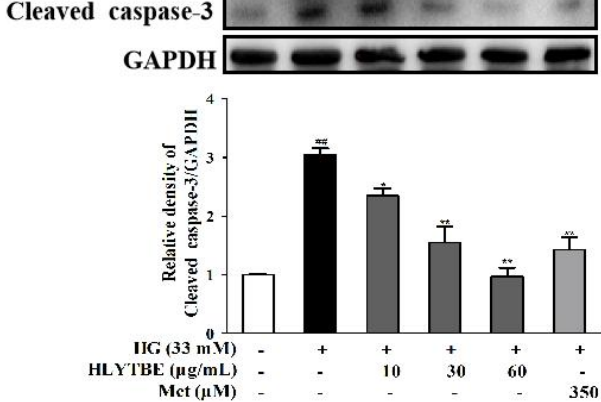
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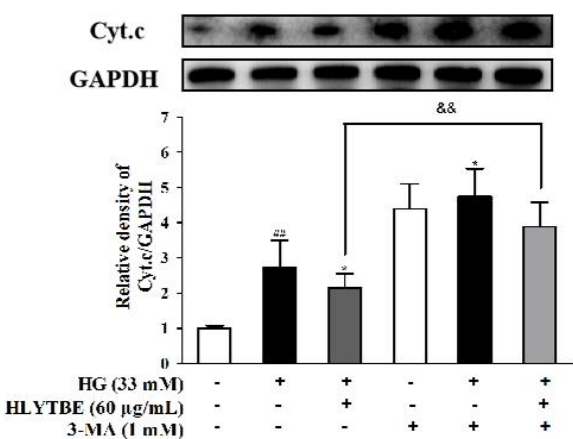
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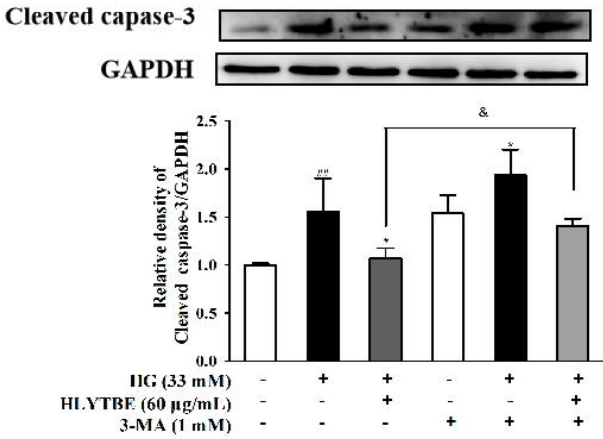
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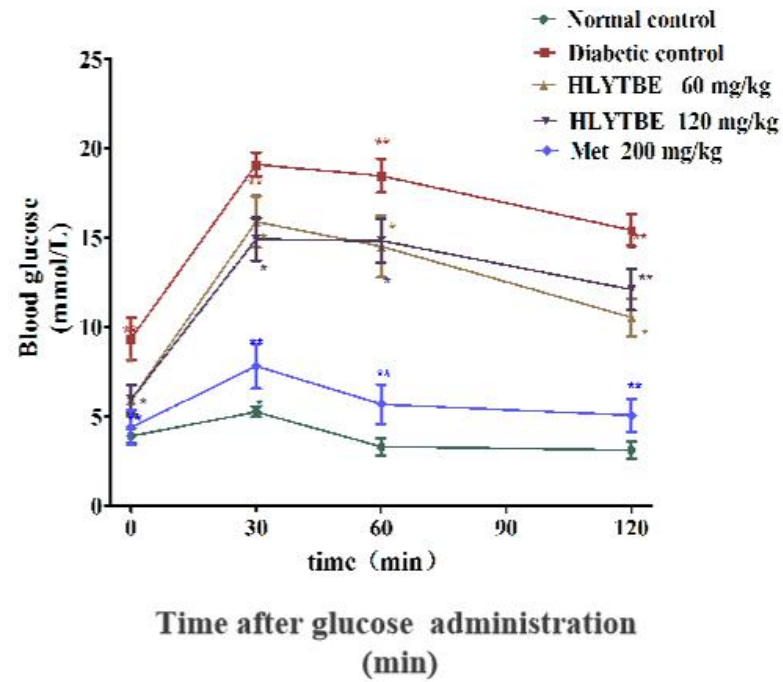
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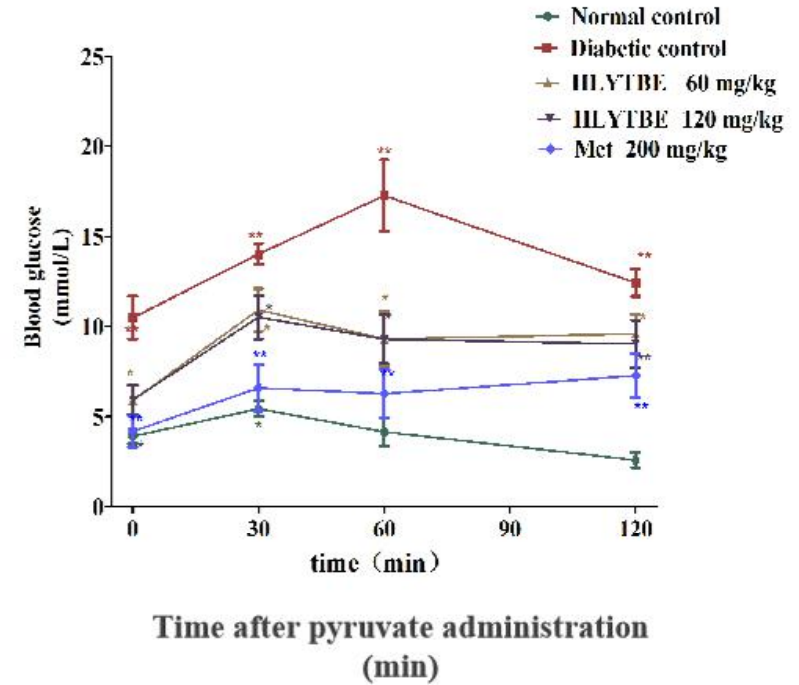
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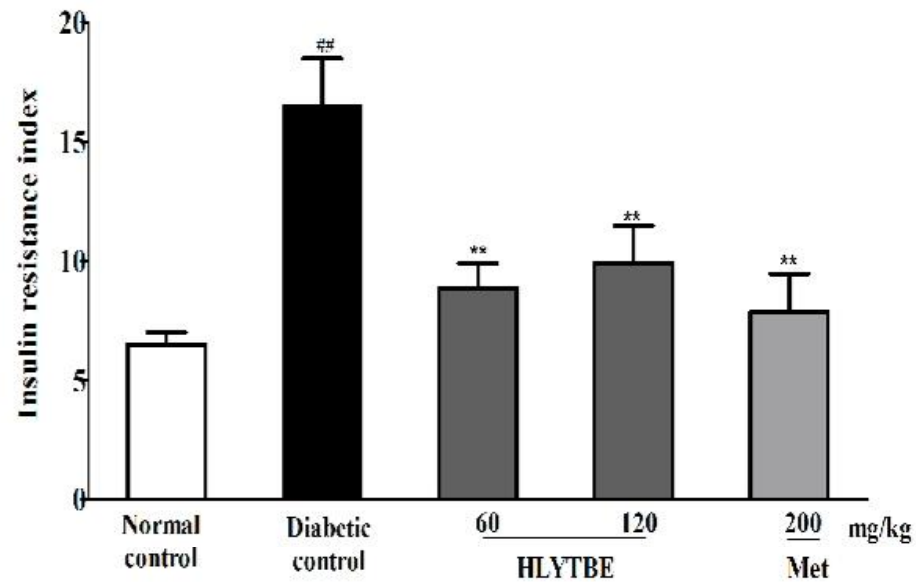


Figure 6

