Glucocorticoids Play a Pivotal Role in Analgesic and Blood Glucose Regulatory Effects in C57 Mice Treated by Water Wxtract of *Prunella Vulgaris* L. Spica

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Research

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Abstract

Background

Prunella vulgaris L. has been a traditional Chinese anti-inflammatory medicine for hundreds of years. However, clinic evidence indicates that it also causes a cluster of metabolic syndromes such as reduced appetite, diabetes, diarrhea etc. To date, the mechanisms of the anti-inflammation and associated symptoms by *P. vulgaris* treatment remain unclear.

Methods

Network pharmacology studies using TCMSP and Genecards database, Cytoscape, GO and KEGG were applied to reveal the ingredients, hub molecules and their interactions, and enriched biologic processes associated with analgesic and glucose intolerance. Some active gradients and target genes were obtained from the comparisons of relevant databases in TCMSP and Genecards. Connections of validated and predicted gradients, target genes and diseases were visualized through Cytoscape. GO and KEGG were applied to identify the significantly engaged hub genes, biological processes and signaling pathways. To further validate GC and BDNF effects, C57BL/6J male mice were randomly divided into 5 groups, control (C), dexamethasone (Dex, 1 mg/kg/day), PE-treated (35mg, 70mg), and PE (70mg) + mifepristone (2.5 mg/kg/day) (PEM group). Mice were pretreated by water extract of *P. vulgaris* spica (PE) for 3-4 weeks followed by one of the following tests: acetic acid-induced writhing, hot plate test, rotaroad test, food intake, glucose tolerance test (GTT). Quantitative PCR was applied to detect hepatic and hypothalamic gene expression. Plasma brain-derived neurotrophic factor (BDNF), glucocorticoids, IL1β, IL6 and IL10 were measured by ELISA.

Results

Network pharmacology studies revealed that BDNF, GCs and GC-responsive or down-stream genes such as GC-induced leucine zipper protein (GILZ), glucose-6-phosphatase catalytic subunit 1, protein kinase B (PKB), etc. were intensively involved into anti-inflammation and glucose intolerance. Acetic acid-induced writhing and hot plate tests confirmed the peripheral and central analgesic effects of PE treatment. Based on the results of feeding behavior tests, 4-week PE treatment impeded food intake but increased the ratio of bodyweight gain to food intake. GTT revealed PE treatment impaired glucose disposal in mice. Finally, real time PCR confirmed that hepatic GC-target genes, such as G6Pase, GILZ, SGK1, PKB were up-regulated, and hypothalamic neuropeptide Y (NPY) and agouti-related protein (AGRP) expression were decreased by PE administration. Glycogen synthase kinase 3 beta (Gsk3 β) was mildly increased. Hypothalamic BDNF was up-regulated, whereas hepatic BDNF was down-regulated. Plasma BDNF and GCs were increased, and IL1β, IL6 and IL10 were decreased by PE treatments ($p<0.05$).

Conclusions

GCs, BDNF, SGK1, insulin and PKB are most relevant molecules to analgesic and glucose intolerance when *P. vulgaris* is applied. PE treatment plays the analgesic role through the GC, GILZ and PKB regulatory pathways, and regulates the levels of some pro-inflammatory cytokines. Meanwhile, it upregulates G6PC1and GSK3β

expression to increase plasma glucose level leading glucose intolerance. Although PE treatment decreases food intake, it makes mice to be prone to obesity. The corresponding increase of plasma BDNF may act as a counterpart to GC effects leading animals to adapt environments more easily.

Introduction

Prunella vulgaris L. is a perennial plant of labiatae family. It is named by its broad range of beneficial effects including immune modulation, anti-viral, anti-allergy, anti-inflammatory, and antioxidant activity [1–7], and its wide distribution over the world [3]. Studies using aqueous or organic fraction from *P. vulgaris* revealed similar effects such as anti-allergy [8], anti-microbial activity [1, 2], and anti-inflammation [3]. Aqueous extracts of *P. vulgaris* (PE) was also reported to inhibit infection [9]. In addition, *P. vulgaris* is used together with other herbal medicine like *Pinellia ternate* (Thunb.) for some classic decoctions in Chinese medicine for dispersing hard lumps and abscess in tissues [10], or as an over-the-counter medication for hypertension [11]. For example, Xia Sang Ju formula, a Chinese drug and herbal tea made up of Prunellae spica, Mori folium, and Flos Chrysanthemi Indici, has commonly used for fever, headache, and sore throat since 1814 [11].The aerial part of *P. vulgaris* is directly used as tea in some areas in China to relief a variety of ailments as well [12].

The widespread use of *P. vulgaris* indicates that it may have numerous components, and/or some potent components to activate critical regulatory pathways. To date, chemical analysis found that *P. vulgaris* contains at least 60 active constituents including Oleanolic-acid, rosmarinic-acid, rutin, ursolic-acid, tannins volatile oil, beta-carotene etc. existing in the root, stem, leave or spica [2–5, 13]. These components can be obtained from either aqueous, ethanol/ methanol extract, but not limited to any single extract [1–3, 13]. The identification of compounds facilitates to postulate them with 86 related diseases, and some of them have been validated (https://tcmsp-e.com). For example, oleic acid stimulates glucagon secretion glucose-independently [14], and involves into brain-derived neurotrophic factor (BDNF) activity [15], which is one of the main factors associating with metabolic regulatory function [16]. Ursolic acid may bind to members of the glucocorticoid receptor to activate GC-signaling pathways [17]. Similarly, rosamarinic acid, a featured compound in plants of labiatae family, is able to attenuate repetitive viral infection stress through up-regulation of glucocorticoid receptor [18, 19]. In addition, oleanolic acid-28-O-beta-D-glucopyranoside and Δ7-stigmasterol in *P. vulgaris* are able to interact with glucocorticoid receptor though the effects and mechanisms remain unknown.

GCs are well known as stress hormones generating an increased blood glucose level to provide enough energy rapidly to respond to the environmental challenge, meanwhile, producing analgesic, anti-nociceptive and depressive and many other therapeutic effects [20]. They are widely used in clinical practice, and have positive effects on control of inflammation, metabolism, and mediation of immune response at physiological concentrations. Prolonged GC administration is often accompanied by severe side effects characterized by the development of diabetes and iatrogenic Cushing syndrome, increasing in risk for serious opportunistic infections, drug resistance and immune system disorders [21]. Mouse models of localized adipocyte-specific GC excess develop visceral adiposity and insulin resistance [22]. Supportive evidence is also obtained from

patients carrying N363S polymorphism of GR with glucocorticoid hypersensitivity, who have a higher body mass index, as well as an increased incidence of obesity and type 2 diabetes [23].

Side effects of clinic use of *P. vulgaris* have been gradually recognized by some physicians though they are not well documented. These side effects include the loss of appetite, weakness, diarrhea after applied for several weeks. Some patients may have immune system disorders, or a gradual loss of kidney/liver function (private correspondence with Professor Shuke Cui, Henan University of Chinese Medicine). To our knowledge, there was limited research on the causal factors(s) and underlying mechanisms for these side effects. Our preliminary studies using network pharmacology approaches revealed that at least 547 genes are relevant to both analgesic and glucose intolerance, only 6 gradients in *P. vulgaris* are identified to target 8 molecules associating with analgesic effect, and 6 components targeting to 9 molecules relevant to glucose intolerance. Therefore, more investigations on the analgesic effect and metabolic regulation of *P. vulgaris* will facilitate to better understand the underlying mechanisms.

Current investigation adopted network pharmacology approaches to predict the involved molecules including GCs, GC-responsive genes, BDNF and some other factors when aqueous extract of *P. vulgaris* spica was applied for anti-inflammatory purpose. The anti-inflammatory effect and impairment of glucose disposal of PE treatment were confirmed by behavioral tests. The expression of involved critical molecules during the treatment was measured by quantitative PCR and ELISA. The study revealed that PE treatment significantly increased plasma GC level, which up-regulated GILZ and G6PC1 for anti-inflammation and glucose intolerance respectively. Together with SGK1, GSK3β, and PKB, the elevated plasma BDNF may fine tune the therapeutic and side effects to lead the animal to be more flexible in the environments. Therefore, the investigation may open the door to better understanding the broad range of beneficial effects of *P. vulgaris*, and assist to develop better strategies for the future use of the herb.

Materials And Methods

Reagents and apparatus

The assay kits for evaluation of the plasma IL1β, IL6 and IL10 were purchased from Wuhan Fine Biotech Co., Ltd (Wuhan, China). Mouse GC and BDNF ELISA Kits were obtained from Biosamite Biotechnology Co. Ltd. (Shanghai, China). Dexamethasone, mifepristone (RU-486) and 2-hydroxypropyl-beta-cyclodextrin were obtained from Sigma Aldrich. Glucose was obtained from Tianjin Kemiou Chemical Reagent Co. (Tianjing, China). Acetonic acid was a product from Fengchuan Chemical reagent Technologies Co. (Tianjing, China). Trizol reagents were sourced from Invitrogen (US). RevertAid First strand cDNA synthesis kit was obtained from Thermal Scientific (US). Primers (Table 1) were ordered from Synbio Technologies (Suzhou, China). All chemicals and reagents were of analytical grade.

Table 1
Primers Used in Realtime PCR

Gene	Forward primer	Reverse primer	Accession #
β-actin	CGGGACCCTGACTATGGGA	GCACTTGCTGAAGTATCGATGG	NM_007393.2
G6pc	GCTGGAGTCTTGTCAGGCAT	ATCCAAGCGCGAAACCAAAC	NM_008061.4
SGK-1	TACCAGACATTCAGCCTCGC	CATGCATAGGTGTTGCTGGC	NM_001161850.2
GILZ	CCTATCCTATAGCGGCAGCG	CGGTGTTCATGGTTCGGTTG	AF024519.1
NPY	TCAGACCTCTTAATGAAGGAAAGCA	GAGAACAAGTTTCATTTCCCATCA	NM_023456.3
AGRP	CAGAAGCTTTGGCGGAGGT	AGGACTCGTGCAGCCTTACAC	NM_007427.3
BDNF	TACCTGGATGCCGCAAACAT	AGTTGGCCTTTGGATACCGG	AY057908.1
GSK3β	CAGTGGTGTGGATCAGTTGG	ACCTTTGTCCAAGGATGTGC	NM_019827.7

YLS-1A apparatus was a product from Yiyan Technology (Jinan, China). Rotarod apparatus (ZB-200) was obtained from Chengdou Technology& Market (Chengdou, China). Microplate Readers was made by BioTek Instruments (Vermont, USA). 2720 thermal cycler was a product of Life Technologies (Applied Biosystems, US). Realtime PCR was performed using ViiA 7 Real-Time PCR System from Thermal Fisher Scientific (US).

Network pharmacology studies

Modified network pharmacology approaches were adopted from our previous work[24] to predict the mechanisms of anti-inflammatory and metabolic side effects of *P. vulgaris*. Briefly, the target molecules and related diseases were obtained from https://tcmsp-e.com. Genes associating with analgesic and/or glucose intolerance were acquired from the database in Genecards. A list of common genes that simultaneously involved in analgesic and glucose intolerance was attained by a comparison of the two sets of genes. A list of validated target genes relevant to the effect of *P. vulgaris* on both analgesic and glucose intolerance was extracted by a comparison of *P. vulgaris* target genes with the list of analgesic and glucose tolerance common genes. Genes with high relevant scores and had not been studied, like BDNF and glucocorticoid signaling molecules, were picked up for this investigation. The expression of these molecules was then confirmed in experimental studies. Together with experimental evidence, a visualized construct showing the connection of PE gradients and target genes associating with the therapeutic effect and side effects was drawn using Cytoscape. An updated protein-protein interaction network (PPI) exhibiting the connections of the genes was produced using https://string-db.org/ database. The obtained PPI data was applied for GO analysis to demonstrate the frequency of the molecules in the network, and identify the hub genes. KEGG analysis was applied to show the significantly enriched signaling pathways for the gene content in the genome provided by KEGG PATHWAY database.

All data cleaning and the comparisons were performed using R associating packages. Values of $P < 0.05$ and $q < 0.05$ were considered to be significant.

Experimental Animals

Health C57 black mice (8 wk old, bodyweight 23 ± 2g) and food were obtained from the Charles River Laboratories (Beijing). Mice were kept in a 12:12 hr light-dark cycle (6:00 AM to 6:00 PM), and fed with standard commercial chow and distilled water *ad libitum*. The room temperature was maintained at 22 ± 0.5 ^{0}C and about 40% relative humidity. The experimental protocols were approved by the Animal Experimentation Ethics Committee at Nanyang Institute of Technology under the guidelines of the National Medical and Health Research Council of China. All protocols were designed to minimize animal discomfort. Only naive mice were used for behavioral tests unless it was pointed out.

Preparation of P. vulgaris **water extract and animal treatment**

PE preparation was made according to the description by Mohaddese et al. [2]. *P. vulgaris* was cultured in the campus of Nanyang Institute of Technology. Spica were obtained and dried when mature during 2018–2020. Three gram dried spike of *P. vulgaris* was ground, soaked in double distilled water (200ml) for 1 hour, and then incubated in 95^{0}C water bath for 2 hours followed by filtering through whatman No.1 filter paper. To avoid the interference from intragastrical administration to behavioral tests, mice were fed with PE at two different concentrations *ad libitum*. According to the daily drink, the daily intake of spike was about 70 mg per mouse in PE70 group, and 35 mg for each animal in PE35 group.

After acclimatizing to the environment for 3–5 days, mice were tested using hot plate assay to get rid of animals with abnormal nociception before randomly divided 5 groups: control (Con. fed by water), PE35 (fed with 35mg/day), PE70 (fed with 70mg/day), PE70 + RU-486(PE + M) (2.5 mg/kg/day, oral) and dexamethasone treatment (Dex) (1 mg/kg/day) groups (n = 8). PE35, PE70 and PE + M groups were pretreated with PE35 or PE70 respectively for two weeks before the application of mifepristone (RU-486). Water soluble Dex was applied two days before experiment and the last one was usually 1 hour before tests. The non-toxic solubilizer, 2-hydroxypropyl-beta-cyclodextrin was dissolved with water to serve as negative control.

Acetic acid induced writhing test

Peripheral analgesic effect of the aqueous extracts of *P. vulgaris* was evaluated using the acetic acid-induced writhing method. 0.6% acetic acid was made from 37% acetic acid with saline. Mice were intraperitoneally injected with 0.1 mL/10 g of 0.6% acetic acid solution 21 days after PE treatments, and 1h after the administration of Dex (1 mg/kg) or saline. Thereafter, the mice were observed for concave abdomen, extension of trunks and hind limbs and lifting of buttocks, indicating abdominal writhing. The number of writhes was counted for 30 minutes after 5 minutes of writhing induction. The percentage inhibition of writhing was calculated relative to control group.

Hot plate test of thermal nociception

Central analgesic activity of *P. vulgaris* was evaluated using hot plate test. The test was performed during 8-10AM using YLS-1A apparatus. Mice were habituated to the testing room for overnight. Mice were then placed in a Plexiglas cylinder on a hot plate at 52^{0}C. Signs of nociceptive behavior were defined as shaking or licking of one of the hind paws or jumping. The latency to lick the hind paw with a 60s cut-off latency was recorded.

Rotarod test

Rotarod task was applied to assess whether or not PE affects motor coordination and balance. Mice were trained twice for up to 5 min before data was collected (spaced 1 h apart to minimize fatigue, 15 and 30 rpm for training, and 30 rpm for testing). The time the animal spent on the rotating rod during each trial was recorded. The rotarod test was carried out 21 days after PE treatment. The chambers were cleaned with water between trials.

Food intake and body weight study during treatment

Food intake and body weight study were followed as our previous description [25, 26]. Briefly, mice were individually housed in regular cages. Body weight and food intake were determined every three days through the experiments during 8-10AM. Pre-weighed food was placed in the food hoppers and measured on a per-cage basis. Food intake was expressed as grams consumed per day. The body weight gain during the experiment was determined by subtracting the body weight right before experiment.

For appetite test, after a 21-day treatment, mice were fasted overnight with control solution or PE available. Body weight and food intake were recorded at 0.5, 1, 4 and 24 hours after food was returned. Average food intake and body weight gain of each group were expressed. The ratio of body weight to food intake was calculated for the indicator of calorie disposal as body weight.

Glucose tolerance test (GTT)

The IPGTT was performed 21 days after PE feeding. After overnight fasting, plasma glucose from tail vein was measured using One Touch Ultraeasy Glucose Test Strip (Shanghai, China) (time 0). Then, animals were injected with an intraperitoneal injection (2g/kg) of glucose. Tail vein glucose was measured at 15, 30, 45, and 60min after injection. Plasma glucose levels were normalized to these of time 0, and reported as relative change (%). Areas under the curves were calculated using Graphpad Prism 8.0.

Blood sampling and ELISA tests for GC, BDNF and IL1β, IL6, IL10

After a three-week treatment, periorbital blood samples was taken and placed in ice-cold EDTA containing tubes, followed by a 3000rpm centrifuge for 30min. Supernants were transferred into well-labeled clean tubes, and saved in -80^{0}C freezer until ready for tests. Plasma levels of IL1β, IL6, IL10, BDNF and GC were measured using mouse ELISA kits respectively according to the manufacturer's instructions. OD values were detected at 450nm.

RNA isolation, reverse transcription and real time PCR

After behavioral tests, mice were anesthetized using ketamine/xylazine (72/13 mg/kg, i.p.). The liver and the hypothalamus were collected. Total RNA was isolated using Trizol method according to the manufacturer's instructions. Reverse transcription reaction, target cDNA quantification and data analysis were performed as the description in our previous work [16, 25] .

Real time PCR was performed in duplicates using 50ng cDNA for each well. Changes in mRNA expression level was normalized to beta-actin level and calculated using the ΔΔCt method. Results were expressed as relative fold change ± SEM.

Statistics

Analysis of difference was performed using one- or two-way ANOVA. When ANOVA reached significance, the t-test was used to compare the mean values among the different groups. A value of $P < 0.05$ was considered to be significant. Data was plotted using Graphpad Prism 8.0 and presented as means ± SE.

Results

Active ingredients in P. vulgaris, **target molecules and enriched biological processes relevant to analgesic and glucose intolerance**

To understand the relationship between anti-inflammation and metabolic dysregulation during PE treatment, common genes of analgesic and glucose intolerance were extracted from the database in Genecards. A total of 2954 in glucose intolerance-related module and 1069 in analgesic-related module were yield respectively, among which 547 genes were their common genes (data not shown). That is to say, 51.2% analgesic-related genes are associated with the occurrence and/or development of glucose intolerance. However, only 8 out of 547 genes appeared as validated *P. vulgaris* target genes in TCMSP database, namely GCG, CCK, PYY, RHO, THBD, INS, TYR, MPO (Fig. 1A). The range of their relevance scores for glucose intolerance was 79.3(INS)-3.4(RHO), and 5.1(CCK)-0.44(THBD) for analgesic. These target genes were associated with only 6 out of the 60 compounds in *P. vulgaris*, including oleic acid, rutin, quercetin, palmitic acid, p-coumaric acid and luteolin (Fig. 1A). Herein, oleanolic acid and its derivatives may have therapeutic effects on different diseases and symptoms, including inflammation [17]. Stigmasterol, a steroid alcohol, is found in numerous medicinal plants, vegetables, and nuts, was reported to alleviate cutaneous allergic responses in animal models [15].

Beyond the above 8 target molecules, the molecule with highest relevance score for glucose intolerance was glucose-6-phosphatase catalytic subunit 1(G6PC1) (89.1), a rate-limit enzyme for hydrolyzing glucose 6-phosphate resulting in the elevation of plasma glucose level. G6PC1 is primarily expressed in the liver and the kidney, and is up-regulated by glucocorticoids through the induction of the binding with glucocorticoid receptor (GR, or NR3C1) to its upstream glucocorticoid responsive element (GRE) (-231 to -129)[14]. GR is one of the 547 target genes with relevance score as 9.4 for glucose intolerance, and 0.6 for analgesic. GR may be activated by Δ7-stigmasterol and oleanolic acid in *P. vulgaris* suggesting as a possible compound-target molecule bridge for the downstream signaling [15, 17]. GILZ, SGK and GSK are GC-responsive genes[27]; meanwhile, they are also in the module of analgesic-glucose intolerance. Their relevant scores are 2.04, 3.4, and 1.3 for analgesic, 13.4, 37.7 and 13.6 for glucose intolerance respectively, indicating their close relationship with anti-inflammation and glucose regulation. In addition, another very important regulatory molecule, BDNF, is also one of the common genes, with relevance score of 2.6 for analgesic, and 9.3 for glucose intolerance, indicating it may play an important role for anti-inflammation and glucose disposal.

Some important cytokines like IL1β, IL6 and IL10, but not limit to, are common genes too. The above genes are also *P. vulgaris* compound targeting molecules.

By using Cytoscape (version 11.0), a construction of compound–target-disease networks was built (Fig. 1A). Their connections among all involved proteins was visualized using String (version 11.0) (Fig. 1B), and GO enrichment analysis revealed that INS, AKT1, IL6, GCG, NPY, BDNF, GCR, AGRP, CCK and PYY were hub genes within the network (Fig. 1C). These molecules actively involved into 9 biological processes, sc. neuroactive ligand-receptor interaction, PI3K-Akt signaling pathway, FoxO signaling pathway, cAMP signaling pathway, regulation of lipolysis in adipocytes, adipocytokine signaling pathway, insulin secretion, AGE-RAGE signaling pathway in diabetic complications, insulin resistance signaling pathways. Functional analysis using KEGG revealed 13 enriched signaling pathways, shown in Fig. 1D ($p < 0.05$, $q < 0.05$). The above evidence indicates that GCR, BDNF etc. intensively involve in the concurrence of analgesic and regulation of glucose homeostasis.

Analgesic effect of PE treatment and its regulation on plasma cytokines

Peripheral analgesic effect of PE was evaluated through acetic acid-induced writhing test, and expressed as inhibition of writhing frequency and percentage relative to control group. Both aqueous PE35 and 70 significantly reduced the number of writhes compared to control group ($p < 0.05$, Fig. 2A). Their inhibitory percentages were 28.5% and 55.8% respectively (Fig. 2B). The application of mifepritone (2.5 mg/kg/day) diminished PE-initiated analgesic effect to the control group level. Dexamethasone significantly decreased the analgesic response compared with control group ($p < 0.05$). The decrease was about the same amplitude as that of PE70 treatment (Fig. 2A, B).

Central analgesic effect was evaluated using hot plate test. After treatment for 20 days, PE35, PE70 and Dex treated groups had significant increased latencies to lick the hind paw from 18.9 ± 1.46 (control group) to 23.2 ± 1.86, 27.3 ± 1.96 and 28.4 ± 1.1s respectively ($p < 0.05$, $n = 8$). The increase was diminished by the application of mifepristone ($p > 0.05$ vs control group), suggesting the central analgesic effect is glucocorticoids relevant (Fig. 2C).

Rotarod test revealed that both PE35 and PE70 treatments significantly reduced the period the animals remained on the rod after a 20-day treatment compared to the control group ($p < 0.05$). Mifepristone was able to rescue the decrease ($p > 0.05$ vs control) (Fig. 2D), suggesting that PE may have a systemic effect on the animals, and the effect of PE on neuromuscular coordination is through GC signaling.

Plasma IL1β, IL6 and IL10 were tested 3 weeks after treatment to ascertain PE dependency of the inhibition of pro-inflammatory cytokines. IL1βwas inhibited 20.2% and 34.1% by PE35 and PE70 respectively (Fig. 2E) ($p < 0.05$). Consistently, IL6 was 222.3 ± 18.8 (9.6%) and 195.4 ± 14.1pg/ml (20.7%) in PE35 and PE70 groups respectively, and they were significantly lower than that of control group, 246.4 ± 21.6pg/ml (Fig. 2F) ($p < 0.05$). Similarly, IL10 level was down-regulated to 81.9 ± 9.1 (13.2%) and 68.7 ± 6.5pg/ml (27.1%) compared to 94.2 ± 12.6pg/ml in control group (Fig. 2G) ($p < 0.05$). RU486 significantly relief the PE inhibitory effect for

the above cytokines ($p > 0.05$ vs control group) (Fig. 2E, F, G). The above results are consistent with the previous work on the regulatory effect of GCs on plasma cytokines [20].

PE inhibitory effects on body weight gain, food intake and appetite

To determine whether PE treatment altered metabolism, mouse body weight was tracked during the experiment. Body weight gain was significant decreased two weeks after PE treatment compared to the control mice. Body weight of PE70 group increased 3.16 ± 0.13 g, whereas the control group increased 4.51 ± 0.31 g ($n = 8$, p□005). After a 4-week treatment, their body weight comparison became 5.0 ± 0.33 vs 6.34 ± 0.39g ($n = 8$, p□005). Dexamethasone treatment demonstrated a similar trend as PE35 treatment (data not shown). The relative body weight change was plotted as Fig. 3A. Meanwhile, PE70 treated mice appeared less food intake after 2 week treatment compared to control mice ($n = 8$, p□0.05) (Fig. 3B). PE35 treatment had the same trend but with less amplitude compared to PE70 for food intake and body weight (Fig. 3A, B), indicating the decrease is PE dose dependent. Mifepristone treatment recovered both food intake and body weight gain to the same level as the control group (Fig. 3A and 3B).

To further test the PE effect on appetite control, after the 20-day PE treatment as previous described, mice were fasted overnight. Then their food intake was measured 0.5h, 1h, 4h after food was returned. PE35 and PE70 treatments significantly reduced food intake compared with control mice (Fig. 3C), suggesting PE treatment decreases appetite. Correspondently, PE70 and PE35 treated-mice gained less weight ($p < 0.05$, Fig. 3D). However, the ratio of body weight gain to the amount of food intake, which is an indicator of calories deposit as body weight [25, 26], was significantly higher in PE70 group from 0.5h to 4h (Fig. 3E), suggesting PE treatment actually increases the tendency of body weight gain relative to food consumption. This evidence gives an interpretation that short-term glucocorticoid therapy does not result in increased body weight, which occurs during long-term therapy [28].

To detect the underlying mechanism associated with body weight and appetite, gene expression analysis was performed using real time PCR. Two hypothalamic orexigenic molecules, NPY (Fig. 3F) and AGRP (Fig. 3G), were highly decreased to 0.21 ± 0.09 and 0.006 ± 0.285 fold by PE70 compared to control mice ($P < 0.05$), which is consistent to the low appetite. Mifepristone recovered PE inhibitory effect, suggesting the central effect of PE treatment is through GC signaling. Dexamethasone treatment also gave similar effects as PE70 (data not shown). Hypothalamic BDNF analysis revealed that PE35 and PE70 treatments significantly increased BDNF level to 3.45 ± 0.27 and 5.70 ± 0.53 folds respectively compared to control group (Fig. 3H) ($P < 0.05$), which is in line with the down-regulation of hypothalamic NPY and AGRP in current study, and appetite increased by BDNF knockdown shown in our previous work [16].

Impairment of glucose tolerance by PE treatment

To test the effect of PE treatment on glucose metabolism, after the 20-day PE treatment, mice were fasted overnight, and basal blood glucose level was measured followed by IP injection of glucose (1.5g/kg). Plasma glucose was measured every 15min afterwards. Both PE35 and PE70 treatments led to a lower fasting plasma glucose level ($p < 0.05$, $n = 8$) (Fig. 4A). To demonstrate PE effects on glucose disposal, glucose level was normalized to that of starting point of each mouse. Relative levels to the starting point were plotted as in

Fig. 4B, showing blood glucose level in PE35 and PE70 groups surged significantly higher than control group 15 min after glucose injection, and maintained until 30 to 45 minutes afterwards ($p < 0.05$, $n = 8$) (Fig. 4B). The AUCs of PE35 and PE70 group were increased by 115% and 135% respectively compared with control group. The AUC of mifepristone treated group was about identical to the control group (99.5%) (Fig. 4C).

To investigate the underlying mechanism of the over-abrupt of plasma glucose level in PE treated mice, hepatic glucose-6-phosphatase catalytic-subunit-encoding gene (G6PC1) expression in the liver was checked using real time PCR. G6PC1 mRNA level was increased by 1.83 ± 0.27 and 2.78 ± 0.31 folds in PE35 and PE70 groups respectively compared with control mice ($p < 0.05$, $n = 4$) (Fig. 4D). Mifepristone inhibited the PE-induced G6PC1 mRNA level ($p > 0.05$ vs control). The above evidence indicates that PE treatment induces gluconeogenesis in the liver by upregulating G6Pase expression, which leads to the impairment of glucose tolerance. Hepatic BDNF was down-regulated by PE35 and PE70 to 0.70 ± 0.11 and 0.42 ± 0.07 folds respectively ($p < 0.05$) (Fig. 4E).

Elevated plasma GC and BDNF levels and the expression of hepatic GC-target molecules by PE treatment

Measurement of plasma glucocorticoids revealed that GC level in control group was 2.94 ± 0.47ng/ml. PE35 and PE70 treatments significantly increased GCs to 9.1 ± 1.8 and 15.7 ± 2.3ng/ml (Fig. 5A) ($p < 0.05$). Meanwhile, plasma BDNF levels were significantly elevated to 143.7 ± 13.1 and 176.6 ± 18.4 pg/mol by PE35 and PE70 respectively compared to 118.4 ± 13.7 pg/mol in control group ($n = 8$) ($p < 0.05$, $n = 8$). PE70 increased significantly higher than PE35 treatment ($p < 0.05$) (Fig. 5B). Mifepristone administration dramatically diminished PE70 effect to control group level (Fig. 5B, $p < 0.05$).The elevated level of plasma BDNF was consistent with its expression in the hypothalamus (Fig. 3H), and other organs (data not shown), but not the liver (Fig. 4E). The corresponding increase of plasma BDNF and GC level indicates their close interplay and the possible mechanism of the concurrence of analgesic and glucose intolerance when PE is chronically applied.

To further confirm the GC-like effects of PE treatment, the transcriptional expression of GC-target genes, including glucocorticoid-induced leucine zipper (GILZ), serum-and glucocorticoid-induced protein kinase 1(SGK1), Glycogen synthase kinase 3 beta (GSK3 β) and serine/threonine-specific protein kinase (Akt2/PKB), was quantified by real time PCR. Hepatic GILZ, which serves as a mediator of the anti-inflammatory effects of glucocorticoid in a variety of cells [27], was increased by 2.98 ± 0.41 folds in PE70 group ($p < 0.05$) (Fig. 5C). Hepatic SGK1, which accelerates the development of metabolic syndrome including glucose intolerance [7], was highly increased by 15.55 ± 1.43 folds ($p < 0.05$) in PE70 group ($p < 0.05$) (Fig. 5D). Hepatic GSK3 β was mildly but significantly increased 1.4 ± 0.11 ($p = 0.0484$) and 1.78 ± 0.26 ($p = 0.007$) folds by PE35 and PE70 respectively (Fig. 5E). GSK3β was originally found to inactivate glycogen synthase and mediate the development of insulin resistance, then identified as a potential target of the therapy for diseases associated with inflammation [9]. In contrast, Akt2/PKB, another GC-target molecule inhibiting glucose release from hepatocytes and increasing insulin sensitivity [29], was also highly up-regulated by 21.41 ± 2.48 folds in PE70 group (Fig. 5F). The above evidence suggests that PE treatment intensifies GC down-stream signaling in the liver closely relevant to anti-inflammation and glucose intolerance.

Discussion

As a traditional herbal medicine, *P. vulgaris* has been intensively used for a variety of disorders. Herein, anti-inflammation is the most common clinic purpose. Network pharmacology investigation found that only 8 out of 547 common genes for analgesic and glucose intolerance were validated as targets of 6 out of 60 ingredients in *P. vulgaris*. Current investigation confirmed the central and peripheral analgesic effect of *P. vulgaris*, which was carried out by increasing plasma GC level, elevating the expression of anti-inflammatory marker gene GILZ, and down-regulating some inflammatory cytokines as shown by the previous work on GCs [20, 27, 30], Simultaneously, PE treatment increased the expression of G6PC1 and some GC-responsive kinases like SGK1, GSK3β and PKB, which modulate glucose homeostasis and anti-inflammatory process in turn. So, the analgesic effect and impairment of glucose disposal of PE treatment were actually from two different downstream signaling pathways of the same GCR/NR3C1 activation by GCs. These findings are consistent with the previous investigations on GCs [14, 20, 21, 31]. In addition, rotarod test also exhibited the GC-like effect of PE application and the alternation of neuromuscular coordination. Moreover, for the first time, current investigation found that plasma BDNF was significantly elevated by PE treatment. Taken together, current investigation confirmed 12 more genes (Fig. 1A), 12 hub gens including glucocorticoid receptor and BDNF (Fig. 1C) and 13 enriched biological activities (Fig. 1D) were intensively involved into anti-inflammatory process and the concurrence of glucose intolerance. The interaction of the 20 genes associating with analgesic and glucose intolerance was constructed (Fig. 1B). The above results at least partially elucidate the mechanisms of anti-inflammatory effect of *P. vulgaris* and its side effect of glucose intolerance.

The elevated GC content and potentiated downstream signaling may be directly induced by the ingredients in *P. vulgaris*. For example, oleic acid can modulate the activity of glucocorticoid receptor and the feedback regulation [32, 33], which may at least partially interpret the upregulation of GC-responsive gene expression and the increase of plasma GCs by PE treatment. In addition, some phytosteroids, like stigmasterol in *P. vulgaris*, may exhibit anti-inflammatory action via different modes through trans-repression or selective COX-2 enzymes, which has similar pharmacokinetic properties as compared to dexamethasone [34]. Nevertheless, the above suggestions may not exclude that sterols in *P. vulgaris* are precursors in the synthesis of steroid hormones, e.g., glucocorticoids in mice [35]. This was supported by our preliminary study using PE to treat C_3H mice, which have live cancer, to yield less GC response and relatively lower plasma GC level compared with C57 mice (data unpublished). Given the fact that GCs are widely applied in numerous diseases such as allergies, inflammations, rheumatoid arthritis, current investigation provided an interpretation for the application of *P. vulgaris* to a variety of symptoms [1–4, 6, 11]. Hence, *P. vulgaris* may be a natural resource for the replacement of synthetic GCs for the treatment of numerous diseases with lesser side effects. Therefore, further investigation focusing on the analysis of GC analogs in *P. vulgaris* and the activation of GC signaling will be crucial for better understand the GC-like effects of *P. vulgaris*, as well the utilization of herbs of labiatae family.

GCs are commonly used at high doses and for prolonged periods from weeks to months in the treatment of numerous symptoms with some well-known side effects, such as glucose intolerance, diarrhea etc. An empirical one among these is impairment of glucose tolerance, which was observed in current study. This

may be caused by the stimulated glucagon secretion by oleic acid [14], and the elevated expression of G6PC1 by the treatment of GC-analogues in *P. vulgaris* resulting in the escalated gluconeogenesis [21, 36]. Besides, glucose intolerance may be contributed by the upregulation of GC-responsive genes and downstream genes, including hepatic SGK1 and PKB/Akt. Excessive SGK1 expression and activity induced by *P. vulgaris* treatment may participate in the pathophysiology of several disorders, including glucose intolerance, obesity, diabetes, and inflammation [7, 37]. Furthermore, the elevated GSK3 β seen in current exploration may inactivate glycogen synthase mediating the development of insulin resistance, which is in line with the clinic evidence that higher expression and activity of GSK3β in patients with type 2 diabetes, and several GSK-3 inhibitors are in the trial to treat various chronic conditions, including metabolic diseases[38]. Current investigations also found that the *P. vulgaris* treated mice had higher ratio of bodyweight gain vs food intake, which were in harmony with the previous conclusion on GCs for the increase of weight gain with central obesity [21]. Regarding the side effect of diarrhea, GCs are causative factors of chronic watery diarrhea through epithelial sodium channel that contributes to absorption of the cation in the human colon [39]. In addition, the diminished expression of PYY and NPY caused by PE treatment may also lead to diarrhea [39, 40]. Furthermore, patients with disturbance of glucose homeostasis are relatively common of small bowel rapid transit [39]. So, current study may provide certain interpretations for some clinic side effects of *P. vulgaris* administration.

Like GC treatment, some evidence of *P. vulgaris* application in current investigation appears counter-intuitive. For example, as the effector molecules of the stress response, GCs are known to increase the consumption of foods enriched in fat and sugar, but acute stress usually results in decreased eating whereas chronic stress results in increased eating [41]. GCs are known as catabolic molecules, but glucocorticoid excess manifests as visceral adiposity [42]. *P. vulgaris* treatment in current research showed a decreased appetite but an increased ratio of body weight gain to food intake. That is to say, PE treatment produced a counter-intuitive evidence of decreased food intake with the expedience of obesity. Likewise, elevated plasma BDNF level and hypothalamic BDNF expression but down-regulated hepatic BDNF expression occurred in current research. More, *P. vulgaris* treated mice had relative lower fasting plasma glucose level compared with control mice (Fig. 4A), but had a higher surge after glucose injection (Fig. 4B), which is found in nondiabetic and type I diabetic humans with impaired glucose disposal following mild hypoglycemia [43]. The above scenarios may be caused by a cluster of GC-responsive genes with opposite functions. For instance, PE treatment simultaneously increases both SGK1 and Akt2/PKB mRNA level by 15.55 ± 1.43 and 21.41 ± 2.48 folds respectively. Herein, Akt2/PKB inhibits glucose release from hepatocytes, enhances glucose utilization and increases insulin sensitivity[29], whereas, the elevated SGK in the liver potentiates glucose generation, exacerbates diet-induced obesity and metabolic syndrome [7]. Opposing to PKA regulation but cooperating with SGK, the elevated GSK3β, followed by phosphorylation and inactivation of glycogen synthase, acts as a negative regulator in the hormone control of glucose homeostasis [44]. Yet, PE treatment may organ-specifically regulate gene expression to harmonize body functions. For example, PE treatment increased hypothalamic BDNF expression to reduce appetite, but decreased hepatic BDNF expression to facilitate G6Pase expression to fulfil the elevation of blood glucose [45]. To muddy the waters further, PE-induced kinase activity may feedback regulate GC activity through a short-term and the long-term feedback regulation of hypothalamic–pituitary–adrenal axis (HPA). For instance, the elevated PKB expression by PE treatment, in turn, mediates the repression of glucocorticoid-stimulated gluconeogenesis to counter balance GC function,

and produces glucocorticoid resistance for feedback inhibition of the anti-inflammatory effect of GC therapy [36]. Confusingly but importantly, the dynamic change may lead to a variety of coping mechanisms to maintain the small oscillating amounts of GCs and normalize behavioral and physiological functions. Therefore, like GCs, PE increases animal metabolic flexibility to better adapt to environmental stress. This also suggests that more pharmacokinetic and pharmacodynamics studies need to be done for successful implementation of *P. vulgaris* therapy as well as understanding the underlying mechanisms.

However, according to some of the details, PE treatment is somehow distinct from exogenous GC administration. First, corticosterone suppresses the BDNF expression at the mRNA and protein level in some brain areas, leading to antidepressant effect of BDNF against corticosterone-induced depression [46]. Current investigation showed a differential BDNF expression pattern in the hypothalamus and the liver, and plasma BDNF was significantly increased under the condition of highly elevated plasma GCs. The increased BDNF may be a combinatorial consequence of tissue specific regulation from GCs as shown in current research and/or linolenic acid-induced increase of serum BDNF as shown in healthy adult humans [47]. Given the fact that BDNF serves important roles in regulating metabolic homeostasis and nociception, the elevated plasma BDNF and organ-specific regulation of BDNF fine tune for GC effects for the subject to be better assimilated into the environments [48–50]. Second, GCs have long been recognized as a food graving stimuli. In contrast, current research demonstrated a reduced appetite, which is in line with the expression of anorexic genes in the hypothalamus, and the study using a neurogenesis-deficient mouse model [51], indicating a different regulation pattern of *P. vulgaris* treatment from GC application.

Overall, this study unraveled PE-induced GC as a curative mediator for the anti-inflammatory effects of *P. vulgaris* through GILZ and downstream signaling, meanwhile, PE treatment upregulated G6PC1 expression for an enhanced gluconeogenesis. It also enhanced SGK1, GSK1β, AKT signaling and downregulation of hypothalamic orexigenic gene expression. Consequently, PE treatment impaired glucose disposal, impeded mouse body weight gain and decreased mouse appetite, but may raise the risk of obesity. PE treatment also increased plasma BDNF level allowing the subject to better adapt to internal and external condition changes. Therefore, our investigation may help to develop optimal therapeutic strategies of a spectrum of symptoms such as inflammation and pain using *P. vulgaris*, meanwhile, also provide an alternative GC resource for the therapy of hypocorticoid pathologies like Addison's disease, antisocial aggressiveness, atypical depression and so on.

Conclusions

At least 11 compounds of *P. vulgaris* contribute to PE treatment for anti-inflammation through 12 hub genes and 13 relevant biological processes. Elevated GC and its induced GILZ play anti-inflammatory roles. GC-induced G6PC1expression leads to the impaired glucose intolerance. Organ-specific expression of BDNF may fine tune GC systemic effects. Insulin, BDNF, IL6, PKB, GC, GCG, NPY and AGRP are the hub genes involving analgesic and glucose intolerance when PE is applied, delineating the mechanisms of analgesic effect of *P. vulgaris* and the concurrence of side effect of glucose intolerance.

Abbreviations

AGRP: Agouti-related protein; AUC: are under curve; CCK: Cholecystokinin; Con: Control; Dex: Dexamethasone; GCs: glucocorticoids; GCG: glucagon; GR, or NR3C1: glucocorticoid receptor; GRE: glucocorticoid responsive element; GILZ: Glucocorticoid-induced leucine zipper; GO: Gene ontology; GSK3 β: Glycogen synthase kinase 3 beta; GTT: glucose tolerance test; HPA: Hypothalamic–pituitary–adrenal axis; IL1: Interleukin 1; IL-6: Interleukin-6; IL-10: Interleukin-10; INS: Insulin; KEGG: Kyoto encyclopedia of genes and genomes; KEGG: Kyoto Encyclopedia of genes and genomes; M: Mifepristone; MPO: Myeloperoxidase; NPY: Neuropeptide Y; PCR: Polymerase chain reaction; PE: Water extract of *P. vulgaris* (spica); PPI: protein-protein interaction network; PYY: Peptide YY; PKB/Akt: Serine/threonine-specific protein kinase; RHO: rhodoposin; SGK1: Serum and glucocorticoid induced kinase 1; THBD: Thrombomodulin; TYR: tyrosinase;

Declarations

Ethics approval and consent to participate

All procedures were performed in accordance with the guidelines of the Nanyang Institute of Technology ethics committee.

Consent for publication

Not applicable.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZZ, LG, CL and CZ designed the studies; QL, XP, GS and WC carried out the experiments; ZZ, PH conducted Network pharmacology study; LG, CZ, CL and ZZ performed the data analysis; LG, CL and ZZ provided manuscript preparation. All authors read and approved the final manuscript.

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References

1. Komal S, Kazmi SAJ, Khan JA, Gilani MM. Antimicrobial activity of Prunella Vulgaris extracts against multi-drug resistant Escherichia Coli from patients of urinary tract infection. Pak J Med Sci. 2018;34:616–20.
2. Mohaddese M, Mahboubi A, Kazempour N. The antimicrobial activity of Prunella vulgaris extracts2015.
3. Rasool R. Prunella vulgaris L. A Literature Review on its Therapeutic Potentials. Pharmacologia. 2013;4:441–8.
4. Bai Y, Xia B, Xie W, Zhou Y, Xie J, Li H, et al. Phytochemistry and pharmacological activities of the genus Prunella. Food Chem. 2016;204:483–96.
5. Wang SJ, Wang XH, Dai YY, Ma MH, Rahman K, Nian H, et al. Prunella vulgaris: A Comprehensive Review of Chemical Constituents, Pharmacological Effects and Clinical Applications. Curr Pharm Des. 2019;25:359–69.
6. Qiu H, Zhang J, Guo Q, Zhang Y, Zhong X. Prunella vulgaris L. attenuates experimental autoimmune thyroiditis by inducing indoleamine 2,3-dioxygenase 1 expression and regulatory T cell expansion. Biomedicine pharmacotherapy = Biomedecine pharmacotherapie. 2020;128:110288.
7. Sierra-Ramos C, Velazquez-Garcia S, Vastola-Mascolo A, Hernández G, Faresse N. Alvarez de la Rosa D. SGK1 activation exacerbates diet-induced obesity, metabolic syndrome and hypertension. J Endocrinol. 2020;244:149–62.
8. Shin TY, Kim YK, Kim HM. Inhibition of immediate-type allergic reactions by Prunella vulgaris in a murine model. Immunopharmacol Immunotoxicol. 2001;23:423–35.
9. Lee JS, Chae MK, Kikkawa DO, Lee EJ, Yoon JS. Glycogen Synthase Kinase-3β Mediates Proinflammatory Cytokine Secretion and Adipogenesis in Orbital Fibroblasts from Patients with Graves' Orbitopathy. Investigative ophthalmology & visual science 2020;61:51.
10. Zhao X, Zhao J, Hu R, Yao Q, Zhang G, Shen H, et al. Ruanjian Sanjie decoction exhibits antitumor activity by inducing cell apoptosis in breast cancer. Oncol Lett. 2017;13:3071–9.
11. Peng M. Network Pharmacology Analysis Uncovers the Potential Anti-Hypertensive Mechanisms of Xia Sang Ju Granule. Journal of Exploratory Research in Pharmacology. 2020;2020:1–10.
12. Liu Y, Ahmed S, Long C. Ethnobotanical survey of cooling herbal drinks from southern China. J Ethnobiol Ethnomed. 2013;9:82.
13. Mojab F, Kamalinejad M, Ghaderi N, Vahidipour H. Phytochemical Screening of Some Species of Iranian Plants. Iranian Journal of Pharmaceutical Research 2010;Volume 2:77–82.
14. Vander Kooi BT, Onuma H, Oeser JK, Svitek CA, Allen SR, Vander Kooi CW, et al. The glucose-6-phosphatase catalytic subunit gene promoter contains both positive and negative glucocorticoid response elements. Molecular endocrinology (Baltimore Md). 2005;19:3001–22.
15. Antwi AO, Obiri DD, Osafo N, Essel LB, Forkuo AD, Atobiga C. Stigmasterol Alleviates Cutaneous Allergic Responses in Rodents. BioMed research international. 2018;2018:3984068.
16. Zhang Z, Zhang Y, Wang Y, Ding S, Wang C, Gao L, et al. Genetic knockdown of brain-derived neurotrophic factor in the nervous system attenuates angiotensin II-induced hypertension in mice. J

Renin Angiotensin Aldosterone Syst. 2019;20:1470320319834406.

17. Ayeleso TB, Matumba MG, Mukwevho E. Oleanolic Acid and Its Derivatives: Biological Activities and Therapeutic Potential in Chronic Diseases. Molecules (Basel, Switzerland) 2017;22.
18. Makhathini KB, Mabandla MV, Daniels WMU. Rosmarinic acid reverses the deleterious effects of repetitive stress and tat protein. Behav Brain Res. 2018;353:203–9.
19. Rahbardar MG, Amin B, Mehri S, Mirnajafi-Zadeh SJ, Hosseinzadeh H. Rosmarinic acid attenuates development and existing pain in a rat model of neuropathic pain: An evidence of anti-oxidative and anti-inflammatory effects. Phytomedicine: international journal of phytotherapy phytopharmacology. 2018;40:59–67.
20. Brattsand R, Linden M. Cytokine modulation by glucocorticoids: mechanisms and actions in cellular studies. Aliment Pharmacol Ther. 1996;10(Suppl 2):81–90. discussion 1–2.
21. Rahimi L, Rajpal A, Ismail-Beigi F. Glucocorticoid-Induced Fatty Liver Disease. Diabetes metabolic syndrome obesity: targets therapy. 2020;13:1133–45.
22. Vandewalle J, Luypaert A, De Bosscher K, Libert C. Therapeutic Mechanisms of Glucocorticoids. Trends Endocrinol Metab. 2018;29:42–54.
23. Serfling G, Buades-Rotger M, Harbeck B, Krämer UM, Brabant G. The corticosteroid prednisolone increases amygdala and insula reactivity to food approach signals in healthy young men. Psychoneuroendocrinology. 2019;99:154–65.
24. Hao P, Xiong Y, Wu H, Yang Y. Network Pharmacology Research and Preliminary Verification of Gegen Qinlian Decoction for the Treatment of Non-Alcoholic Fatty Liver Disease. Natural Product Communications. 2020;15:1934578X20920023.
25. Zhang Z, Liu X, Morgan DA, Kuburas A, Thedens DR, Russo AF, et al. Neuronal receptor activity-modifying protein 1 promotes energy expenditure in mice. Diabetes. 2011;60:1063–71.
26. Fernandes-Santos C, Zhang Z, Morgan DA, Guo DF, Russo AF, Rahmouni K. Amylin acts in the central nervous system to increase sympathetic nerve activity. Endocrinology. 2013;154:2481–8.
27. Ronchetti S, Migliorati G, Riccardi C. GILZ as a Mediator of the Anti-Inflammatory Effects of Glucocorticoids. Front Endocrinol (Lausanne). 2015;6:170.
28. Berthon BS, MacDonald-Wicks LK, Wood LG. A systematic review of the effect of oral glucocorticoids on energy intake, appetite, and body weight in humans. 34. New York: Nutrition research; 2014. pp. 179–90.
29. Mackenzie RW, Elliott BT. Akt/PKB activation and insulin signaling: a novel insulin signaling pathway in the treatment of type 2 diabetes. Diabetes metabolic syndrome obesity: targets therapy. 2014;7:55–64.
30. Bereshchenko O, Migliorati G, Bruscoli S, Riccardi C. Glucocorticoid-Induced Leucine Zipper: A Novel Anti-inflammatory Molecule. Front Pharmacol. 2019;10:308.
31. Pariante CM. Why are depressed patients inflamed? A reflection on 20 years of research on depression, glucocorticoid resistance and inflammation. Eur Neuropsychopharmacol. 2017;27:554–9.
32. Viscardi RM, Max SR. Unsaturated fatty acid modulation of glucocorticoid receptor binding in L2 cells. Steroids. 1993;58:357–61.

33. Pegoraro NS, Camponogara C, Cruz L, Oliveira SM. Oleic acid exhibits an expressive anti-inflammatory effect in croton oil-induced irritant contact dermatitis without the occurrence of toxicological effects in mice. J Ethnopharmacol. 2021;267:113486.
34. Marahatha R, Gyawali K, Sharma K, Gyawali N, Tandan P, Adhikari A, et al. Pharmacologic activities of phytosteroids in inflammatory diseases: Mechanism of action and therapeutic potentials. Phytotherapy research: PTR; 2021.
35. Aboobucker SI, Suza WP. Why Do Plants Convert Sitosterol to Stigmasterol? Frontiers in plant science. 2019;10:354.
36. van Schaftingen E, Gerin I. The glucose-6-phosphatase system. Biochem J. 2002;362:513–32.
37. Lang F, Stournaras C. Serum and glucocorticoid inducible kinase, metabolic syndrome, inflammation, and tumor growth. Hormones (Athens). 2013;12:160–71.
38. Gupte M, Umbarkar P, Singh AP, Zhang Q, Tousif S, Lal H. Deletion of Cardiomyocyte Glycogen Synthase Kinase-3 Beta (GSK-3β) Improves Systemic Glucose Tolerance with Maintained Heart Function in Established Obesity. Cells 2020;9.
39. Camilleri M, Sellin JH, Barrett KE. Pathophysiology, Evaluation, and Management of Chronic Watery Diarrhea. Gastroenterology. 2017;152:515–32. e2.
40. Moriya R, Shirakura T, Hirose H, Kanno T, Suzuki J, Kanatani A. NPY Y2 receptor agonist PYY(3–36) inhibits diarrhea by reducing intestinal fluid secretion and slowing colonic transit in mice. Peptides. 2010;31:671–5.
41. Hewagalamulage SD, Lee TK, Clarke IJ, Henry BA. Stress, cortisol, and obesity: a role for cortisol responsiveness in identifying individuals prone to obesity. Domest Anim Endocrinol. 2016;56(Suppl):112-20.
42. Lewis T, Zeisig E, Gaida J. Does glucocorticoid exposure explain the association between metabolic dysfunction and tendinopathy? Endocrine connections. 2020;9:R36–46.
43. Davis MR, Shamoon H. Impaired glucose disposal following mild hypoglycemia in nondiabetic and type I diabetic humans. Metab Clin Exp. 1992;41:216–23.
44. Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature. 1995;378:785–9.
45. Guzzardi MA, Sanguinetti E, Bartoli A, Kemeny A, Panetta D, Salvadori PA, et al. Elevated glycemia and brain glucose utilization predict BDNF lowering since early life. J Cereb Blood Flow Metab. 2018;38:447–55.
46. Schaaf MJ, De Kloet ER, Vreugdenhil E. Corticosterone effects on BDNF expression in the hippocampus. Implications for memory formation. Stress. 2000;3:201–8.
47. Hadjighassem M, Kamalidehghan B, Shekarriz N, Baseerat A, Molavi N, Mehrpour M, et al. Oral consumption of α-linolenic acid increases serum BDNF levels in healthy adult humans. Nutrition journal. 2015;14:20.
48. Jeanneteau F, Chao MV. Are BDNF and glucocorticoid activities. calibrated? Neuroscience. 2013;239:173–95.

49. Marosi K, Mattson MP. BDNF mediates adaptive brain and body responses to energetic challenges. Trends Endocrinol Metab. 2014;25:89–98.
50. Yang X, Liu S, Zhang Z. Sex difference in blood pressure, a combinatorial consequence of the differential in RAAS components, sex hormones and time course. Current hypertension reviews 2021.
51. Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature. 2011;476:458–61.

Figures

Fig 1

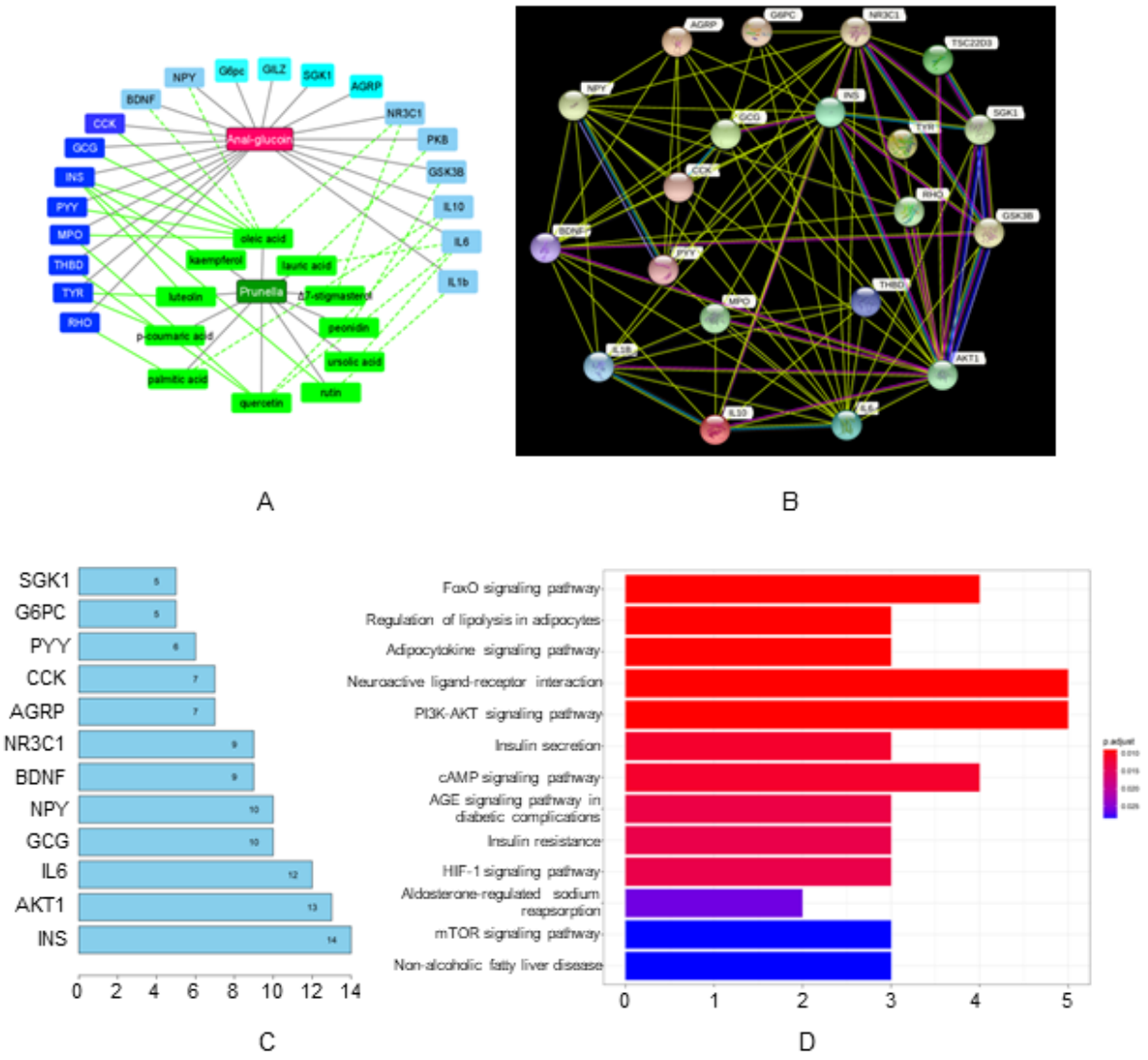

Figure 1

Mechanisms of the anti-inflammatory effect and concurrence of glucose intolerance of P. vulgaris administration. A. Construction of compound-target molecule-disease network, visualizing current validated ingredient–target interaction (dots in royal blue; edge, solid grey) and predicted connection information (dots in aquamarine for molecules that were able to connect with P. vulgaris ingredients; dots in cyan represents molecules that not directly connect to ingredients in current database; edge, dot grey); P. vulgaris ingredients shown in light green; Prunella shown in dark green: anal-glucoin represents analgesic and glucose intolerance, shown in red. B. A PPI network showing current validated target molecules (inner circle dots) and predicted molecules (outer circle dots) by current investigation, where nodes and edges represent proteins and their interactions. C. The degrees of hubs (≥ 5) in network of Fig 1B. D. KEGG analysis showing significantly enriched signaling pathways of the validated and predicted proteins ($p<0.05$, $q<0.05$). The calibration scale represents the adjusted p value.

Fig. 2

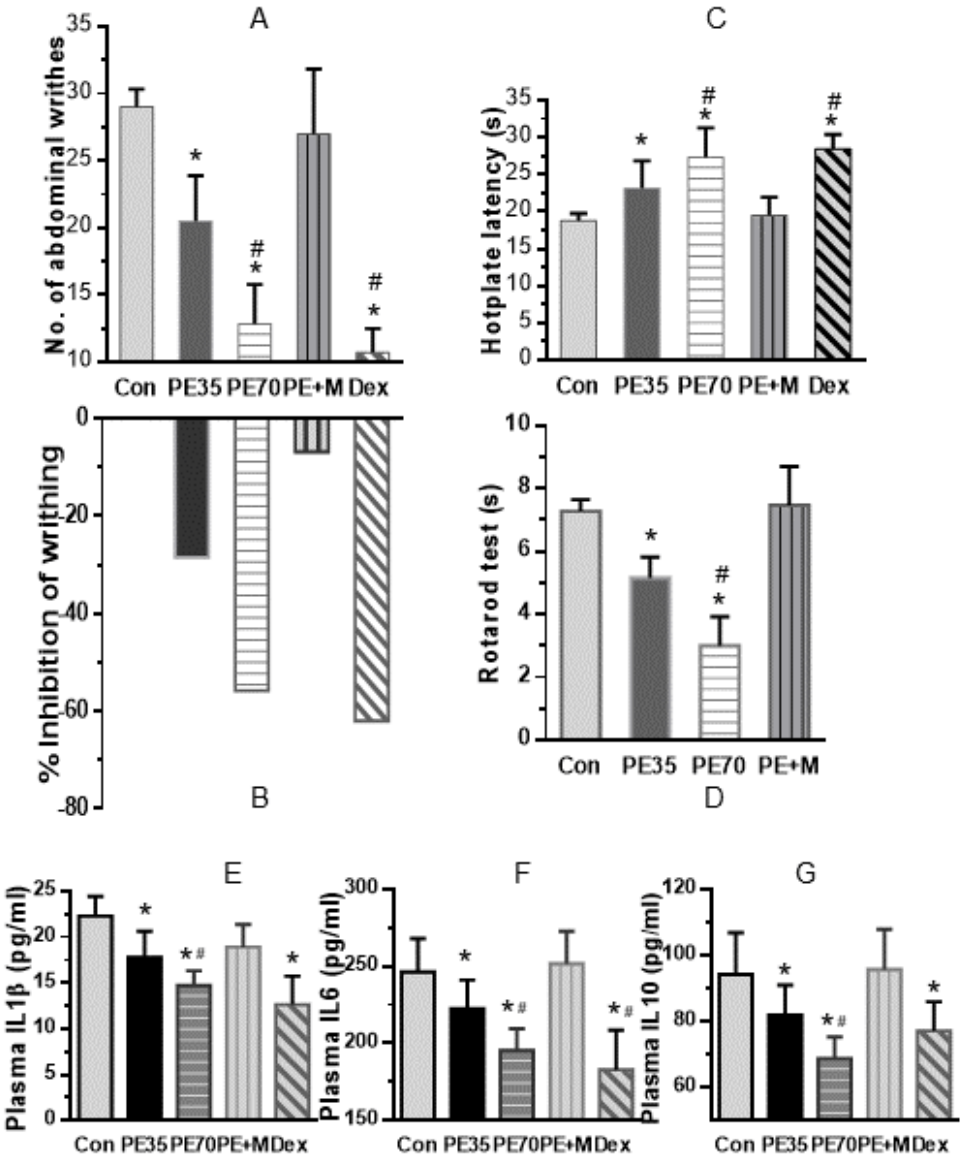

Figure 2

Behavioral modification and plasma cytokine regulation of PE. A. Peripheral analgesic effect of PE using acetic acid-induced writhing test showing in counts (A), and inhibitory rates (B). C. Central analgesic effect of PE using hot plate test. E-G: PE effect on the level of plasma cytokines IL1β (E), IL6 (F) and IL10 (G). Data was plotted as mean ± SE. Con: Control; PE: P. vulgarias water extract; M: mifepristone. *p□0.05 vs control group; # p□0.05 vs PE35 (n=8).

Fig. 3

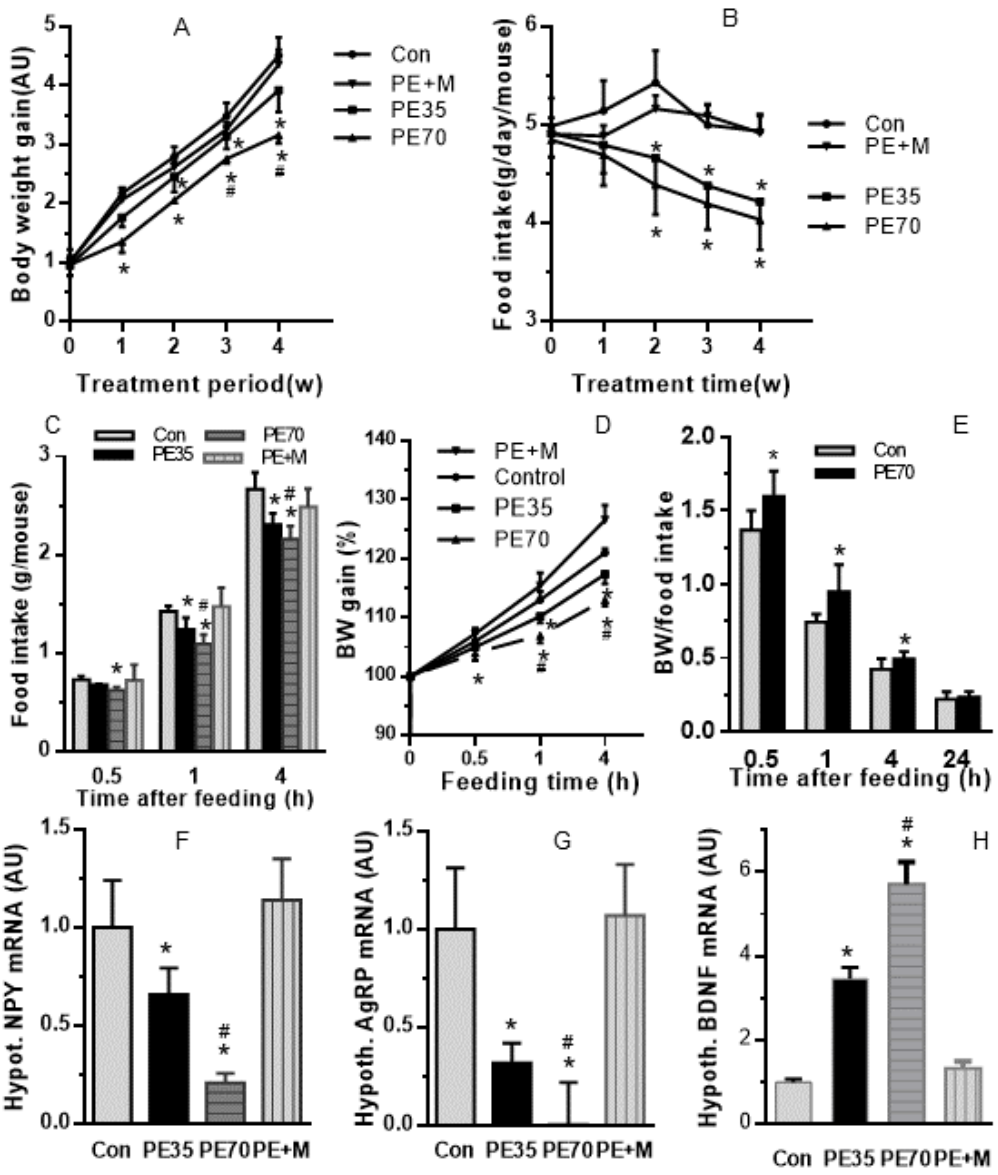

Figure 3

The regulation of PE on food intake, body weight gain, and hypothalamic gene expression. A.B. Food intake using individual host cage showing PE35 and PE70 significantly decreased body weight gain (A), and food intake (B). C. Fasting food intake at 0.5, 1 and 4 h after food return. D. Relative body weight gain during fasting food intake test. P70 and P35 significantly decreased body weight gain. E. Comparison of the ratio of weight gain (%) to food intake in control and PE70 groups showing PE70 treatment increases the ratio at 0.5, 1 and 4 h after food return. F. PE effect on hypothalamic NPY expression; G. PE effect on hypothalamic

AGRP expression. H. Decreased hypothalamic BDNF expression by PE treatment. PE: P. vulgaris water extract; Con: control; Data was plotted as mean ± SE. *p<0.05 vs control group; # p<0.05 vs PE35 (n=4-8).

Fig. 4

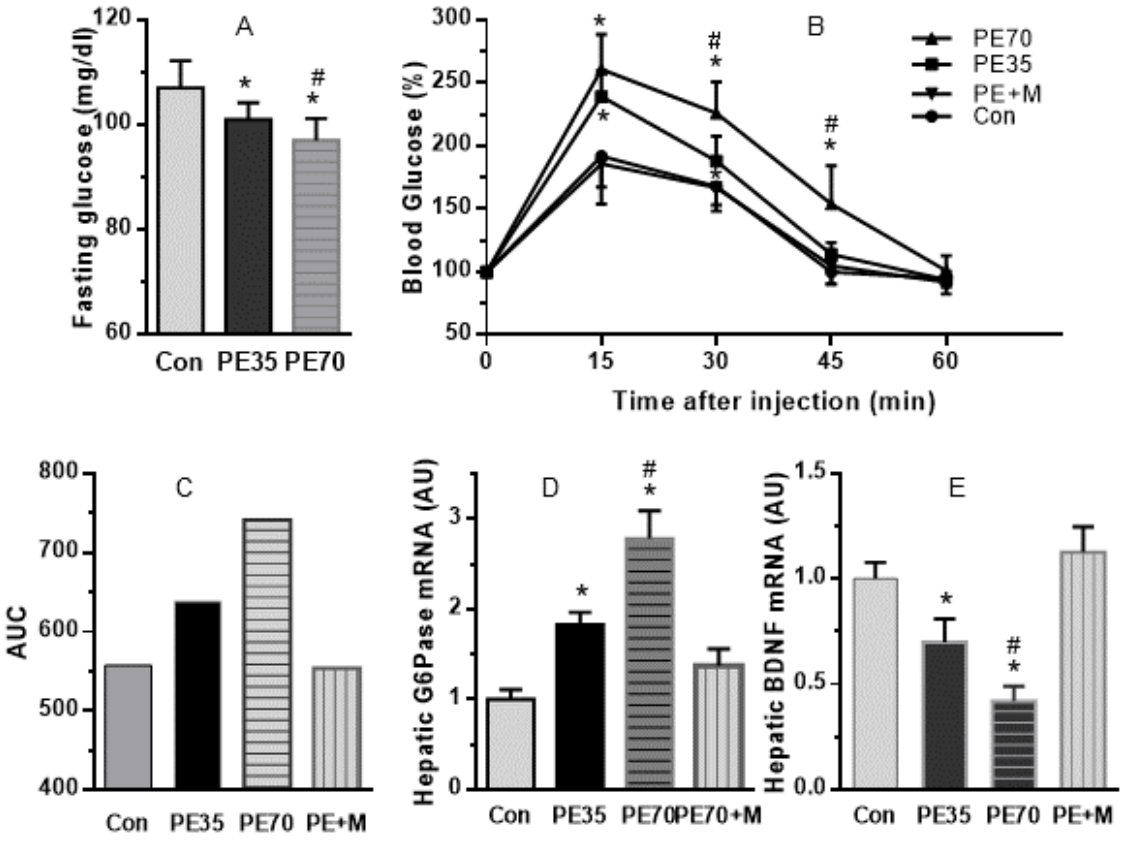

Figure 4

PE effect on glucose metabolism and hepatic gene expression. A. Fasting glucose level showing decreased fasting blood glucose level by PE treatment. B. Dynamic change of relative blood glucose level showing increased blood glucose levels 15 min after IP injection by PE35 and PE70 treatments. C. Quantification of the area under curve in B. D. Regulation of hepatic G6PC1 expression by PE35 and PE70 treatments. E. Regulation of hepatic BDNF expression by PE treatment. Data was plotted as mean ± SE. *p<0.05 vs control group; # p<0.05 vs PE35 (n=4-8).

Fig 5

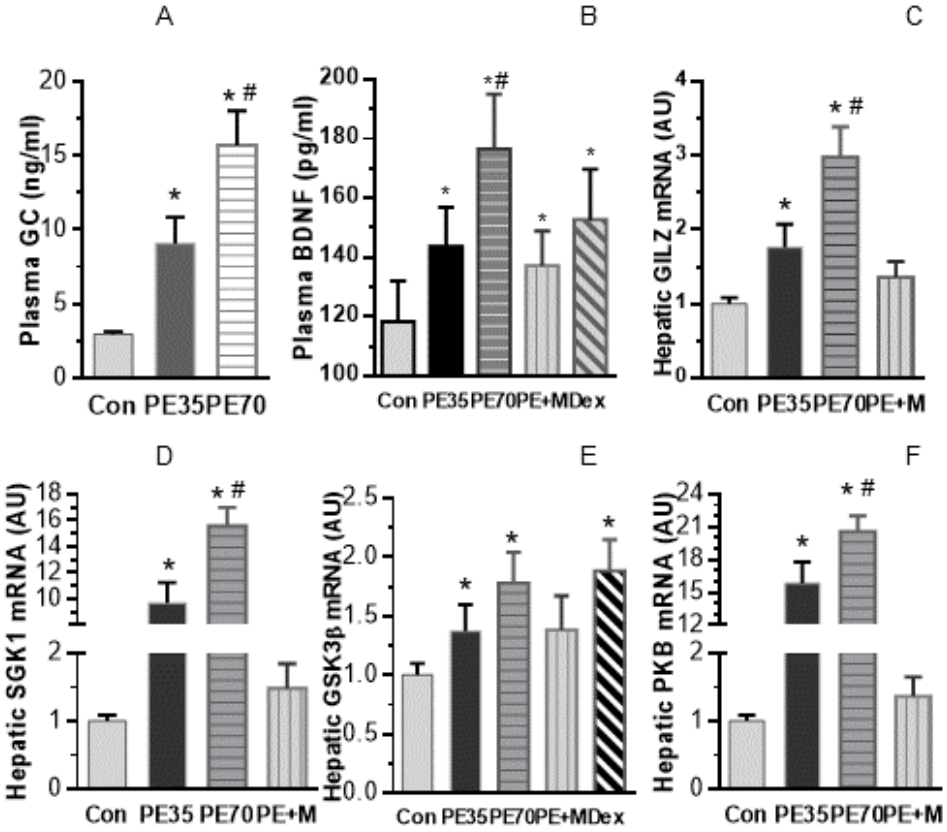

Figure 5

Influence of PE treatment on plasma GC (A) and plasma BDNF (B) level (n=8), hepatic GILZ (C), hepatic SGK1 (D), GSK3β (E) and PKB (F) expression(n=4). Data was plotted as mean±SE. * indicates $p<0.05$ when vs Con. ; # p□0.05 vs PE35.