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Research Article

Codonopsis javanica root extracts attenuates hyperinsulinemia and lipid peroxidation in fructose-fed insulin resistant rats

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ABSTRACT

From ancient times, Dǎngshēn (*Codonopsis javanica*) has been used in Chinese traditional medicine. In this study we investigated the anti-hyperinsulinemia and antioxidant properties of *C. javanica* root extracts in a rat model of insulin resistance (IR), induced by chronic fructose feeding. Twenty-four Sprague–Dawley rats were randomized into control, fructose-treated (10%, w/v), and fructose then *C. javanica* (Fru + Cod)-treated groups. After 8 weeks fructose feeding, increased fasting serum insulin levels (2.6 ± 0.45 μg/L) and insulin area under the curve confirmed the IR ($p < 0.001$). However, *C. javanica* treatment to fructose-fed rats significantly attenuated the hyperinsulinemia with correspondingly improved glucose tolerance. Weight gain in Fru + Cod group was comparably ($p < 0.01$) lower than in the fructose-fed group. Furthermore, IR-induced increased hepatic lipid peroxidation, as demonstrated by elevated malondialdehyde levels, were significantly ($p < 0.001$) alleviated by *C. javanica* treatment. These findings reveal that chronic fructose intake may facilitate IR and oxidative damage, which could be eradicated by improved antioxidant status. Accordingly, we found that *C. javanica* treatment significantly improved the antioxidant enzyme activities, including superoxide dismutase, glutathione peroxidase and glutathione reductase in the liver. These findings that fructose-induced hyperinsulinemia and associated oxidative stress could be attenuated by *C. javanica* root extracts.

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1. Introduction

Increased consumption of fructose-enriched food products is associated with prevalence of metabolic disorders, including weight gain, insulin resistance (IR) and hyperlipidemia in both animals and humans [1,2]. IR represents a cluster of metabolic disorders, such as obesity and glucose intolerance, and predisposes to type 2 diabetes [2,3]. Diabetes-mediated complications are increasing worldwide, including Taiwan [4]. The liver is the primary site for fructose extraction and metabolism, therefore, chronic high fructose load impairs hepatic glucose metabolism [1,5]. Furthermore, administration of fructose can trigger free radical production, thereby decreasing antioxidant status and causing oxidative damage to proteins and lipids in the liver [6–8]. Therefore, antioxidant status in the liver is a major concern when evaluating fructose-induced metabolic syndrome.

Codonopsis javanica is a vital herb in Chinese folk medicine. Extracts of *C. javanica* and other *Codonopsis* species have been used to treat diabetes and other diseases. *C. javanica* belongs to the Campanulaceae family, usually grows under the shade of trees, and produces bell-shaped flowers [8–10]. Root extracts of *Codonopsis* species possess pharmacological efficacies, such as antifatigue [11], antioxidant, antitumor, antimicrobial and immune-boosting properties [12–14]. The pharmacological efficacy of *Codonopsis* roots is most likely due to the various constituents, including polysaccharides, saponins, alkaloids, and phytosteroids [15,16].

The purpose of the present study was to investigate the potential beneficial effects of *C. javanica* root water extracts on hyperinsulinemia and antioxidant status in a rat model of IR. IR was induced by chronic fructose feeding of healthy rats in order to mimic type 2 diabetes mellitus. In addition to glucose tolerance and antioxidant enzyme status, oxidative damage to lipids and proteins was also evaluated in the liver of rats.

2. Methods

2.1. Animals

Twenty-four healthy male adult Sprague–Dawley rats (6 months old) were purchased from the BioLASCO Taiwan Co. Ltd., Taipei Prevalence of fructose-induced metabolic syndrome is more common in adult populations, therefore, we chose adult rats as experimental animals in this study. All rats were maintained in a temperature-controlled ($23 \pm 2\,^{\circ}C$) room with fixed 12-hour light and 12-hour dark cycle. Rats were freely fed a standard laboratory chow (LabDiet 5001; PMI Nutrition International, Brentwood, MO, USA) and water *ad libitum*. The entire study design and protocols were approved by the Animal Ethics Committee, Taipei Physical Education College, and performed according to the guidelines for the Use of Research Animals published by the Council of Agriculture, Executive Yuan, Taiwan.

2.2. Experimental design and treatment

The detailed groups were as follows: Group 1 (control): eight rats were provided with a normal diet and tap water for the 13 weeks of the experimental period; Group 2 (fructose drinking): eight rats were allowed to drink 10% fructose water (w/v) for 13 weeks. IR was confirmed after 8 weeks fructose water feeding based on oral glucose tolerance test (OGTT); and Group 3: fructose plus *Codonopsis* treatment (Fru + Cod): eight rats were fed fructose water as described for Group 2, and were then treated with *C. javanica* root extracts at a dose of 1 mg/kg. *C. javanica* treatment was started after 8 weeks fructose feeding, and continued for 5 weeks along with same concentration of fructose water feeding. According to the animal body weights, *C. javanica* root extract was dissolved in a minimal quantity of drinking water to achieve a dose equivalent to 1 mg/kg of root extract, and allowed to drink freely.

After completion of the final treatments, all rats were sacrificed under anesthesia (chloral hydrate, 400 mg/kg, intraperitoneal), and liver tissues were collected. The tissues were immediately washed with ice-cold saline, excess blood was removed, and tissues were frozen into liquid nitrogen until further biochemical analysis. During the study period, body weight and food and water intake were recorded every 3 days for all groups (data not shown).

2.3. Preparation of plant extracts and dose

The roots of *C. javanica* (Bl.) J.D. Hooker subsp. *Japonica* were purchased from local farmers in Yunlin County, Taiwan. *C. javanica* roots were collected during the winter season, and the age of the plant at the time of collection was about 2 years. Origin of the *C. javanica* was identified by Chao-Lin Kuo, School of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University, Taichung, Taiwan, where a plant specimen was deposited.

The roots were sliced and dried in a circulating air stove (Eyela NDO-600ND, Tokyo, Japan). The dried roots were powdered mechanically, and 10 L of hot water was added to the powder, which was decocted for 4 hours. Water was removed by distillation under reduced pressure, and the remaining content was lyophilized (FreeZone 6 Liter Benchtop Freeze Dry System, Labconco, MO, USA), and stored under light protection to yield crude aqueous deep-brown extract (13.16 %). For the pharmacological tests, lyophilized extract was dissolved in saline solution prior to use.

The preliminary dose-dependent studies from low to high dose (0.1 mg/kg, 1 mg/kg, 10 mg/kg and 100 mg/kg) were performed to evaluate the effective dose for the rats. On the day of the experiment, different concentrations of root extracts were freshly prepared, and administered orally (orogastric tube) to rats 1 hour prior to the OGTT. We found an effective response with 1 mg/kg, which was assayed through OGTT and insulin tolerance test. The calculated glucose area under the curve (GAUC), which was found to be lower with 1 mg/kg dose compared to other doses (0.1 mg/kg, 10 mg/kg and 100 mg/kg), and the same dose was used in the study (Fig. 1).

2.4. Induction of IR in healthy rats

IR in normal rats was induced by regular fructose water feeding (*ad libitum* 10 %, w/v) for a period of 8 weeks. After fructose water drinking, OGTT was performed under fasting

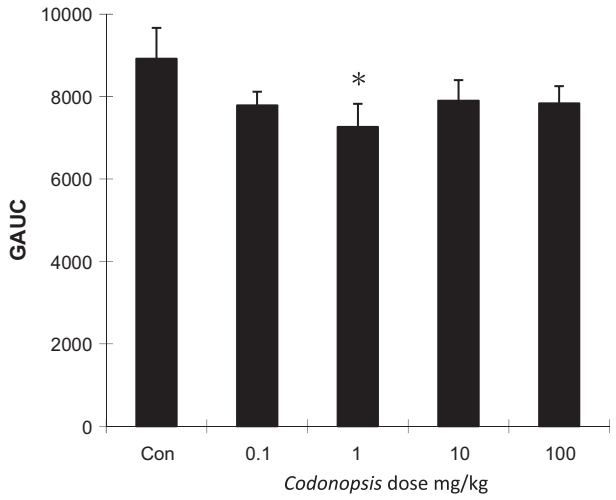

Fig. 1 – Evaluation of effective dose of *Codonopsis javanica* through dose-dependent studies. Con = control; GAUC = glucose area under the curve. *Values are significant compared to other doses of *Codonopsis*.

conditions to determine the glucose tolerance and insulinemia for all groups. Rats with impaired glucose tolerance and higher serum insulin levels (2.6 ± 0.45 μg/L) at Week 8 confirmed the hyperinsulinemia/IR in this study (Table 2), and then IR rats proceeded to the remaining intervention. After confirmation of IR, fructose feeding was continued for a further 5 weeks until the end of the experiments.

2.5. OGTT

In order to determine the oral glucose tolerance and to confirm the IR, an OGTT was performed twice under fasting conditions. The first OGTT was performed after 8 weeks fructose water feeding, and the second was after 4 weeks of *C. javanica* treatment under continuous fructose feeding for 12 weeks. On the day of the OGTT, 50% glucose solution (w/v) was administered to each rat (1 g/kg) via an orogastric tube. Then, blood samples were collected at 0 min (fasting sample) and at 30 minutes, 60 minutes, 90 minutes, and 120 minutes from the tail vein for blood glucose and insulin assays.

At the time of blood collection, individual rats were gently covered with a towel and placed on the experimental table. The tail was gripped and disinfected with an alcohol swab. The tip of the tail was punctured and blood samples were collected into a clean vial. A single drop of fresh blood was placed on a glucose test strip to determine the blood glucose levels. Gentle pressure was applied during the blood collection and special care was taken to avoid any blood vessel damage and infection. The tail was cleaned with an alcohol swab and held for a few seconds to stop bleeding.

2.6. Estimation of fasting blood glucose and serum insulin levels

Blood glucose levels were estimated by a glucose analyzer (Lifescan, Milpitas, CA, USA). For insulin assay, about 200-μL blood samples were centrifuged at 3500 rpm for 10 minutes to obtain serum. The serum insulin levels were quantified on an enzyme-linked immunosorbent assay (ELISA) analyzer (A-5082; Tecan Genios, Salzburg, Austria) using the commercial ELISA kit (Diagnostic Systems Laboratories, Webster, TX, USA) according to the manufacturer's protocol. Based on the OGTT data, GUAC and insulin response curves (IAUCs) were calculated for all groups.

2.7. Evaluation of antioxidant enzyme activities

Liver tissue was homogenized in ice-cold phosphate buffer (50 mM, pH 7.4, containing 0.1 mM EDTA) and centrifuged at 1000 rpm for 10 minutes at 0 °C. The supernatant was used to determine antioxidant enzyme activities. The primary antioxidant enzyme, superoxide dismutase (SOD) activity was assayed as described by Misra and Fridovich [17]. Optical density was measured at 480 nm for 4 minutes, and the activity was expressed as the amount of enzyme that inhibited the oxidation of epinephrine by 50%, which was equal to 1 U. According to Aebi [18], catalase (CAT) activity was monitored with Triton X-100 by measuring the optical density at 240 nm for 1 minute on a UV spectrophotometer (10S UV-Vis; Genesys, Oshkosh, WI, USA). CAT activity was expressed as μmol H_2O_2 degraded per minute per milligram of protein. Glutathione peroxidase (GPx) and glutathione reductase (GR) activities were measured as described by Flohe and Gunzler [19], and Carlberg and Mannervik [20] respectively. For both assays the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) was monitored at 340 nm for 3 minutes in a spectrophotometer. The final activities were expressed as μmol NADPH oxidized per minute per milligram of protein. All enzyme activities were calculated per milligram of protein, and the protein concentration was determined by Bio-Rad protein assay protocol (Richmond, CA, USA).

2.8. Determination of lipid peroxidation and protein oxidation indices

Oxidative degradation of lipids, referred to as lipid peroxidation, was determined by measuring malondialdehyde (MDA) levels in the tissue homogenates, according to the protocol described by Ohkawa et al [21]. Protein oxidation in liver samples was determined by measuring the protein carbonyl residues using 2,4- dinitrophenylhydrazine. This assay was performed according to the protocol provided by Cayman's commercial kit (Ann Arbor, MI, USA), and the amount of protein–hydrozone product was quantified spectrophotometrically at 360 nm on an ELISA plate reader (A-5082; Tecan Genios, Salzburg, Austria).

2.9. Statistical analysis

All the data were calculated and analyzed for significance using SPSS and Instat GraphPad software. Results were expressed as mean ± standard error for eight replicates. One-way analysis of variance was carried out to compare the level of significance followed by Tukey's multiple comparison *post hoc* test. A p value < 0.05 was considered statistically significant.

Table 1 – Effect of fructose and *Codonopsis javanica* treatment on body weight changes during a period of 12 weeks.

Groups	Week 1	Week 8	Week 12	Weight gain (g)
Con	555.3 ± 22.5	597.4 ± 8.5	625.13 ± 27	69.3
Fru	572.25 ± 17	649.62 ± 15.6	686.14 ± 17[a]	113.9
Fru + Cod	570 ± 21	645.4 ± 12.5	651.2 ± 21[b]	81.17

Values are expressed as mean ± standard error.
Con = control; Fru = fructose; Fru + Cod = fructose + *C. javanica*.
[a] Significant compared to 12-week control group ($p < 0.05$).
[b] Significant compared to 12-week fructose-fed group ($p < 0.05$).

3. Results

3.1. *Effect of fructose feeding and C. javanica treatment on body weight*

We recorded body weight regularly from Week 1 to Week 12. Tukey's multiple comparison tests showed that initial body weight was not significantly different among the groups. However, body weight at Week 12 was significantly higher in the fructose-fed group compared to the control group ($p < 0.05$). As shown in Table 1, overall weight gain was greater in the fructose group (113.9 g) than the control group (69.3 g) and *Codonopsis*-treated group (81.17 g) ($p < 0.05$). These data indicate that fructose-induced weight gain was countered by *C. javanica* treatment (Table 1).

3.2. *C. javanica attenuates hyperinsulinemia and impaired glucose tolerance*

Statistical analysis clearly indicated that fasting insulin levels were significantly elevated after 8 weeks fructose feeding (2.6 ± 0.45 μg/L) compared to the control rats (1.5 ± 0.27 μg/L) ($p < 0.01$). This increase reached a maximum (3.89 ± 0.43, $p < 0.001$) with continuous fructose feeding at Week 12 (Table 2). Hyperinsulinemia or IR was confirmed after 8 weeks fructose feeding, as indicated with higher insulin and IAUC values under an OGTT (Fig. 2C and 2D). Impaired glucose tolerance and higher GAUC values further supports the IR condition in fructose-fed rats (Fig. 2B). However, the elevated insulin levels were noticeably suppressed after *C. javanica* treatment in Group 3; this decrease was significant ($p < 0.05$) compared to that in the fructose alone group (Table 2). Furthermore, profoundly elevated insulin levels ($p < 0.001$) during OGTT at Week 12 were also decreased along with reduced IAUC values by *C. javanica* treatment (Fig. 3C and D).

Blood glucose levels estimated every 30 minutes under an oral glucose challenge were significantly higher in fructose-fed rats compared to control rats at Week 8 and Week 12 (Figs. 2A and 3A; $p < 0.01$, $p < 0.05$). These data clearly indicate impaired glucose tolerance, which resulted from chronic fructose feeding. The calculated GAUC data for the fructose group were also higher than in the control group at Week 8 and Week 12, which was partially significant with Tukey's multiple comparison tests (Figs. 2B and 3B). Nevertheless, *C. javanica* treatment improved the glucose tolerance (lower blood glucose), and GAUC values tended to decrease to normal compared to those in the fructose alone group under OGTT (Fig. 3A and 3B).

3.3. *Effects of C. javanica on antioxidant enzyme activities in IR rats*

Prior to *C. javanica* treatment, hepatic SOD activity was significantly lower in IR rats compared to control rats ($p < 0.01$). However, SOD activity was significantly restored to normal levels after *C. javanica* treatment in Group 3 (Fig. 4A; $p < 0.01$).

In contrast to other antioxidant enzymes, CAT activity was significantly increased in fructose-fed rats ($p < 0.05$), but was not significantly altered with *C. javanica* treatment. The increase in CAT activity with fructose drinking might have been a compensatory response to cope with excessive H_2O_2-mediated toxicity in the liver (Fig. 4B). Both CAT and GPx enzymes have functional similarity in removal of H_2O_2. In our study, GPx activity was retained in the fructose group, while CAT activity was increased as aforementioned. Functional similarities of CAT and GPx may have contributed to the stable GPx activity. Nevertheless, we found threefold higher GPx activity in *C. javanica*-treated IR rats. Statistical analysis revealed that increased GPx activity with *C. javanica* was significantly higher when compared to that in the control and fructose alone groups (Fig. 4C; $p < 0.001$).

Similar to the SOD response, liver GR activity was also significantly decreased in IR rats (Fig. 4D; $p < 0.001$). The reduction in GR activity was more pronounced than SOD activity. However, the GR activity was significantly regained by *C. javanica* treatment after the fructose-induced decrease ($p < 0.05$).

Table 2 – Fasting blood glucose and serum insulin levels before (Week 8) and after (Week 12) *Codonopsis javanica* treatment in fructose-fed rats.

	Week 8		Week 12		
	Con	Fru	Con	Fru	Fru + Cod
Glucose (mg/dL)	54.7 ± 3.2	58.7 ± 3.4	53.63 ± 4.8	65.57 ± 6.7	55.38 ± 4.8
Insulin (μg/L)	1.5 ± 0.27	2.6 ± 0.45**	1.46 ± 0.26	3.89 ± 0.43***	2.69 ± 0.26*,#

Values are expressed as mean ± standard error. Values are significant compared to control (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$), and fructose (#$p < 0.05$) groups in their respective weeks.
Con = control; Fru = fructose; Fru + Cod = fructose + *C. javanica*.

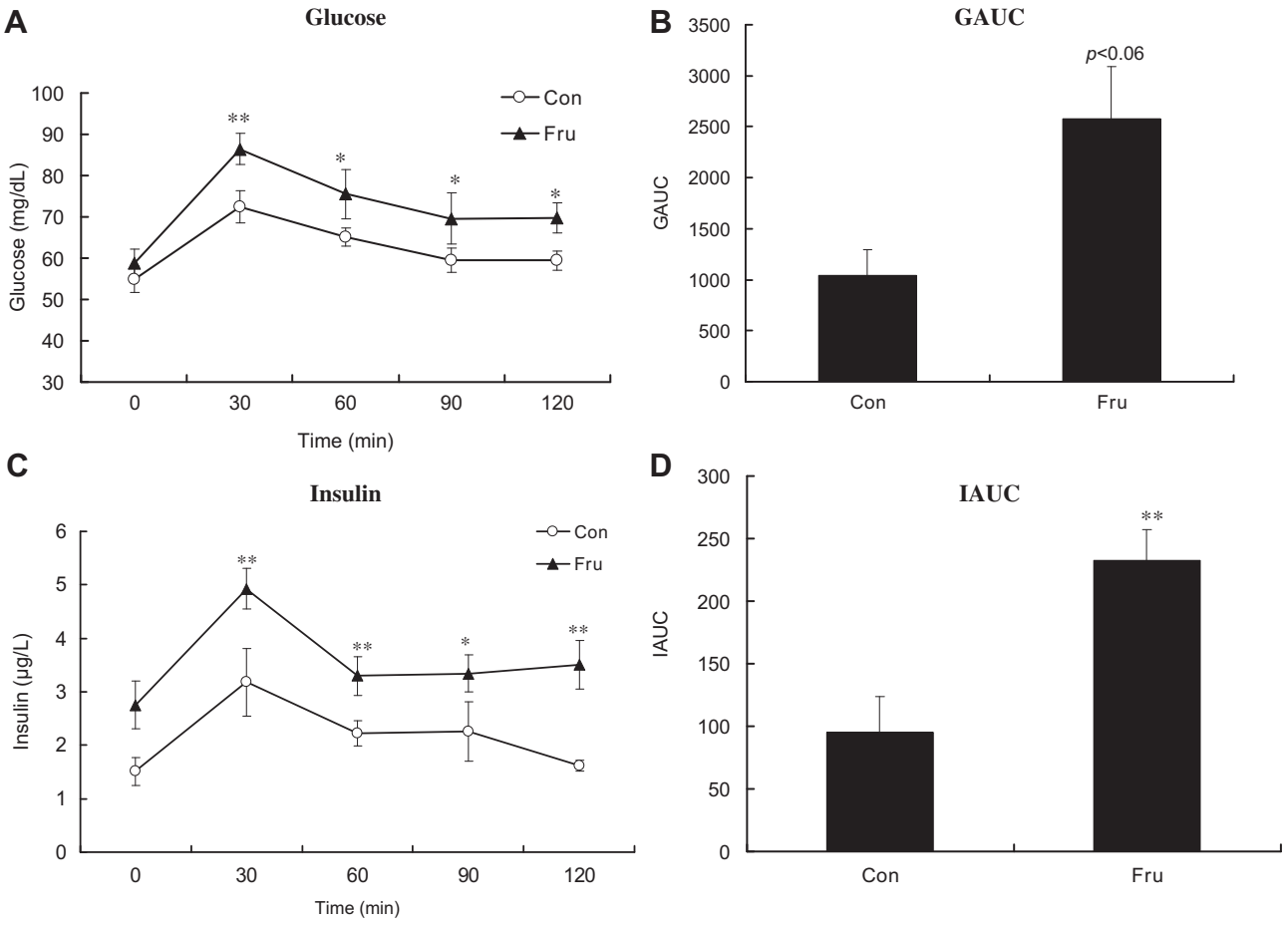

Fig. 2 – Confirmation of insulin resistance after 8 weeks fructose feeding by oral glucose tolerance test. (A) Glucose; (B) GAUC; (C) Insulin; (D) IAUC. Values are significant compared to control (*$p < 0.05$; **$p < 0.01$). Con = control; Fru = fructose; GAUC = glucose area under the curve; IAUC = insulin area under the curve.

3.4. *Effect of C. javanica on fructose-induced lipid peroxidation and protein oxidation*

To address whether *C. javanica* treatment is able to reverse the oxidative damage in the liver of IR rats, lipid peroxidation and protein oxidation indices were determined. Statistical analysis clearly indicated that the lipid peroxidation index, in terms of MDA levels, was significantly elevated with fructose feeding compared to that in normal control rats ($p < 0.01$). IR rats treated with *C. javanica* extract showed significantly lower MDA levels than those in the fructose alone group ($p < 0.01$). It is noteworthy that MDA content in the *C. javanica*-treated group was almost similar to that in the control group (Fig. 5A).

Long-term fructose-consumption-induced oxidative damage to proteins in the liver was clearly demonstrated by elevated protein carbonyl residues in Group 2 ($p < 0.001$). However, the elevated carbonyl residues with fructose feeding were not suppressed completely by 5 weeks *C. javanica* treatment, which may need longer and/or a higher dose (Fig. 5B).

4. Discussion

Hyperinsulinemia and/or IR are closely associated with oxidative stress and liver damage. It has been shown that chronic intake of high fructose diet can cause hyperinsulinemia and oxidative stress in the liver of rats [6,7,22]. In this study, we found that disruption of insulin homeostasis and the antioxidant system with fructose feeding was alleviated by *C. javanica* root extract supplementation. To the best of our knowledge, this is the first report to demonstrate the therapeutic effects of water extracts of *C. javanica* root against chronic fructose-induced oxidative stress in the liver of rats.

Long-term fructose consumption, in terms of increased calorie intake is associated with a greater increase in body weight in humans and animals [2,22]. Fructose feeding increases food intake (data not shown), which may enhance fat deposits and increase body weight over a period of time. Fructose consumption can reduce the circulating leptin levels, which play a key role in regulating food intake and energy expenditure, thus increasing calorie intake [2]. *C. javanica*

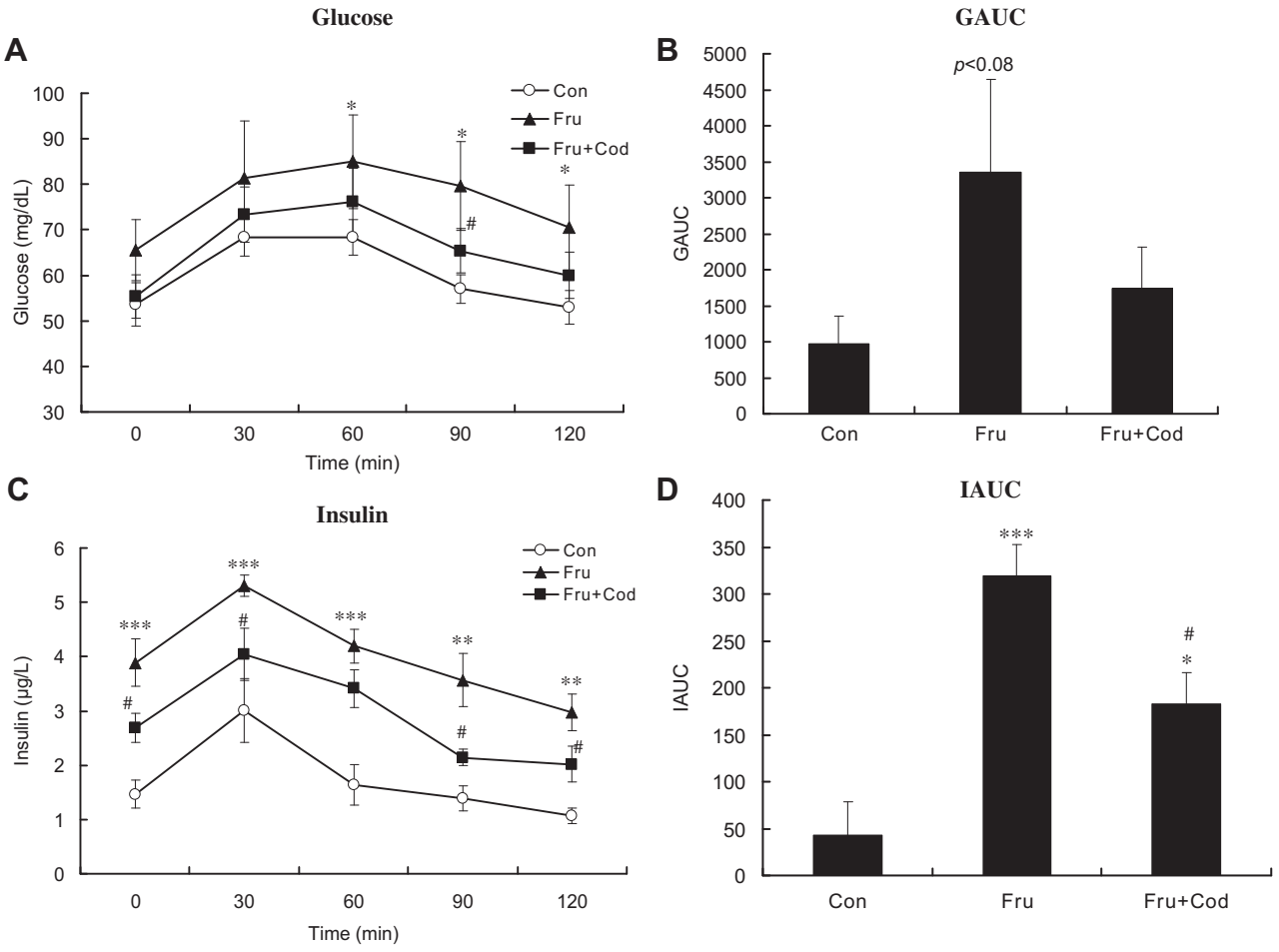

Fig. 3 – Effect of *Codonopsis javanica* treatment on impaired glucose tolerance and hyperinsulinemia. (A) Glucose; (B) GAUC and hyperinsulinemia; (C) insulin; (D) IAUC in fructose-fed rats. Values are significant compared to control (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$) and fructose-fed (#$p < 0.05$) groups. Con = control; Fru = fructose; Fru + Cod = fructose + *C. javanica*; GAUC = glucose area under the curve; IAUC = insulin area under the curve.

treatment along with fructose feeding reduced the overall weight gain, which indicates its weight management effects.

In agreement with previous studies, hyperinsulinemia/IR was observed in fructose-fed rats. It has been shown that rats fed with 60% fructose diet for 60 days exhibit higher insulin and glucose levels [22]. Chronic fructose feeding alters the activities of several enzymes involved in hepatic carbohydrate metabolism, including decreasing glucokinase and increasing glucose-6-phosphatase activities, which lead to IR [23]. Besides, hyperinsulinemia in fructose-fed rats may impairs β-cell function, because the cells cannot cope with increased insulin demand due to IR [7]. Under this circumstance, supplementation of antioxidant substances, such as *C. javanica* extract, decreased the insulin levels. *Codonopsis* mixture with other herbs shows hypoglycemic properties, which may be associated with improved pancreatic β-cell function [10]. Increased oxidative stress is considered as an instigator of IR [24], and decreased oxidative stress and improved hepatic antioxidant capacity by *C. javanica* treatment may be beneficial against the IR caused by fructose feeding.

The antioxidant system plays a crucial role in preventing the progression of nonalcoholic fatty liver [25]. As an antioxidant enzyme, SOD scavenges the superoxide radicals ($O_2^{\bullet-}$) into H_2O_2, which was decreased with fructose feeding in our study. The major reason for SOD reduction could be an increase in $O_2^{\bullet-}$ production and/or glycation of the active site of SOD under hyperglycemic conditions [6,26]. Under these circumstances, liver cells are more prone to oxidative damage or necrosis and lose their original function. Besides, normalizing the $O_2^{\bullet-}$ production has been shown to prevent hyperglycemic damage [27]. Restored SOD activity by *C. javanica* treatment indicates that excessive $O_2^{\bullet-}$ radicals may be effectively eliminated, and hyperglycemia-mediated enzyme inactivation alleviated. A previous study showed that aqueous extracts of *Codonopsis pilosula* (a member of the Campanulaceae) inhibited peroxy-radical-mediated hemolysis in rat erythrocytes [28]. Furthermore, a herbal formulation SR10 that comprises *Codonopsis* extracts increased the hepatic SOD activity in diabetic mice [10]. In our study, we speculate that antioxidant compounds in *C. javanica* extracts may have mitigated the fructose-induced excessive $O_2^{\bullet-}$ radical toxicity and protected the liver by restoring SOD activity.

Liver CAT activity was increased after fructose feeding, which might be part of the defensive response against

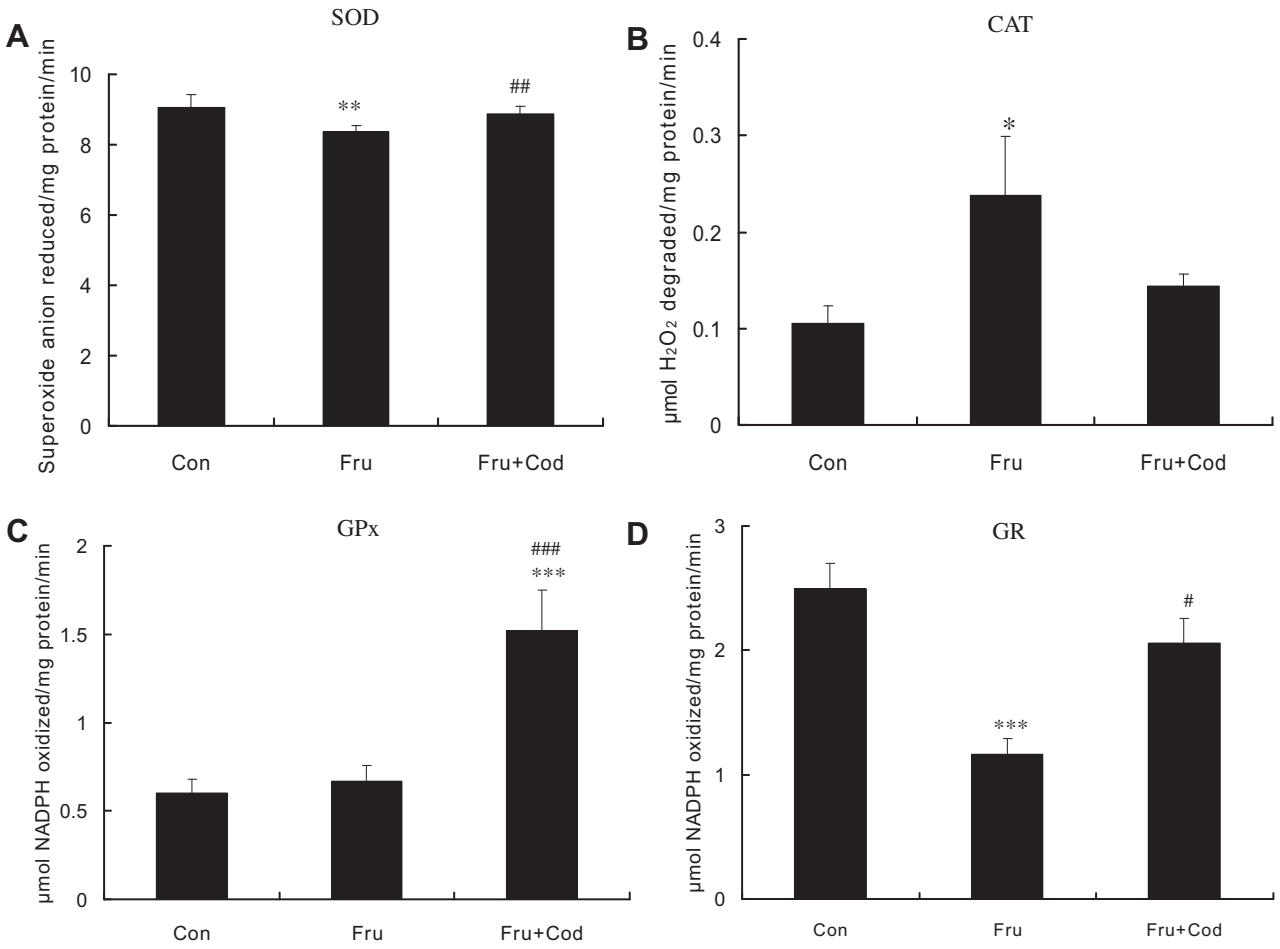

Fig. 4 – Response of liver superoxide dismutase (A), catalase (B), glutathione peroxidase (C) and glutathione reductase (D) activities to *Codonopsis javanica* treatment in insulin resistant rats. Values are significant compared to control ($^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$) and fructose-fed ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$) groups. Con = control; Fru = fructose; Fru + Cod = fructose + *C. javanica*.

fructose-induced oxidative stress. Similarly, Pasko and colleagues [29] reported increased CAT activity in the testes of fructose-fed rats (310 mg/kg, 5 weeks), which implies that CAT is necessary for decomposition of toxic H_2O_2. By contrast, Francini et al [7] reported decreased CAT activity along with unaltered GPx activity with a 10% fructose diet, which may facilitate oxidative stress in rat liver. These findings reveal that fructose can induce oxidative stress, but the CAT response can be divergent in tissues. This discrepancy might have been due to the difference in duration and dose of fructose and/or tissue-specific response. However, under our experimental conditions, we speculate that hepatic cells might have evolved with a defense mechanism, thereby expressing higher CAT activity to cope with fructose-induced, H_2O_2-mediated toxicity. Removal of H_2O_2 typically relies on CAT or GPx activity, therefore, the unaltered GPx in the fructose-fed group implies increased CAT activity. Increased CAT activity may be enough to scavenge the excessive H_2O_2, therefore, GPx activity remains stable. Nonetheless, increased GPx activity with *C. javanica* extracts suggests that active ingredients in root extract boost the GPx activity. The active ingredients, such as polysaccharides and saponin in *Codonopsis* [15,16] may be responsible for its pharmacological effects, including antioxidant activity.

It has been shown that hyperglycemic conditions can increase ROS production through glucose auto-oxidation and protein glycation [30,31]. This phenomenon could block the active sites of many enzymes, including GR, which is more susceptible to inhibition. Blakytny and Harding [32] found a time-dependent inhibition of GR activity in bovine intestine with fructose, suggesting that fructose glycates this enzyme. A particularly interesting finding from our study was that decreased GR activity was effectively restored by *C. javanica*, which indicates that *C. javanica* is effective in ROS elimination and avoids the GR glycation process. Water-soluble polysaccharides and other antioxidant ingredients in *Codonopsis* species [14, 28] may inhibit oxidative inactivation of enzyme molecules. Increased GR activity may further facilitate maintenance of the stable GSH resynthesis cycle.

Excessive production of ROS under IR/hyperglycemic conditions attacks the local cell organelles, including membrane lipids, which results in lipid peroxidation [30,33]. Fructose-

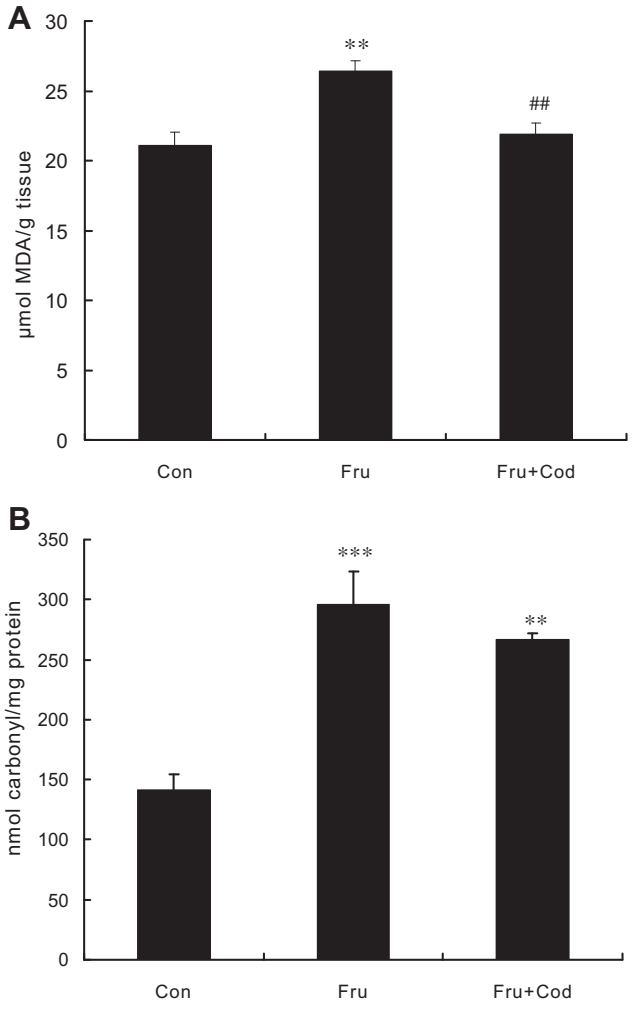

Fig. 5 – Effect of *Codonopsis javanica* treatment on malondialdehyde (A) and protein carbonyls (B) in the liver of insulin resistant rats. Values are significant compared to control ($^{**}p < 0.01$ and $^{***}p < 0.001$) and fructose-fed ($^{\#\#}p < 0.01$) groups. Con = control; Fru = fructose; Fru + Cod = fructose + *C. javanica*.

induced increased oxidative damage was demonstrated by elevated MDA levels in our study. Decreased antioxidant status with fructose feeding may be attributed to increased lipid peroxidation. The key finding of the study was that increased lipid peroxidation was attenuated by *C. javanica* root extracts. It has been demonstrated that root extracts of *Codonopsis* species inhibit lipid peroxidation in the rat brain [28] and raw sheep meat [12]. The phytochemicals and saponins in root extracts may be responsible for the inhibition of lipid peroxidation. Although the molecular mechanism behind this inhibition is unclear, we assume that improved SOD and GR activities might arrest the elongation of the lipid peroxidation process and protect the lipid membranes.

Oxidative damage to proteins imposed by fructose feeding was reflected by elevated protein carbonyl residues in the liver of IR rats. Our results showed slightly reduced protein oxidation with *Codonopsis* supplementation. We assume that > 5 weeks treatment and/or a high dose of *C. javanica* may be necessary to bring about complete reduction. By contrast, fructose feeding was continued, while animals received *C. javanica* extracts. Under fructose feeding, excessive ROS production may interfere with the beneficial effects of *Codonopsis* in the liver.

In conclusion, our results demonstrate that water extracts of *C. javanica* roots could effectively attenuate chronic fructose-induced obesity, hyperinsulinemia, and elevated membrane lipid peroxidation. The therapeutic effects of *C. javanica* were further supported by maintaining the stable antioxidant status in the liver of rats with IR. These findings provide evidence for the medicinal importance of *C. javanica*, which is an important herb in the fields of Chinese traditional medicine and food research. Our study suggests that increase intake of herbal food that possesses antioxidant substance, such as *C. javanica*, may be helpful to prevent/avoid IR-mediated metabolic complications.

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