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# *Euonymus alatus* Extract Reduces Insulin Resistance in db/db Mice by Regulating the PI3K–AKT Pathway

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**Abstract:** In accordance with the usage of *Euonymus alatus* (EA) as folk medicine in diabetes, the present study employed water and 70% ethanol twig extract to assess its antidiabetic effects in C57BL/KsJ-db/db mice. These effects were then compared with those observed in normal C57BL/6J Jms Slc mice. After 4 weeks of supplementation with 70% ethanolic EA extract or water EA extract by oral gavage at a dose of 500 mg/kg with distilled water (DW) per day, body weight was measured and compared with the diabetic group (Db). HPLC demonstrated that the maximum flavonoids were extracted in the Et.EA extract rather than in the water EA extract. The supplementation of the Et.EA extract significantly increased liver and muscle glycogen content with respect to the Db group. Additionally, the Et.EA extract modulated the expression of glycogen synthase (GS) in the liver and muscles of Db mice, indicating that it plays a promotive role in glycogen synthesis. Mechanistically, Et.EA extract activates insulin receptor substrate (IRS1/IRS2)/phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) in the liver and muscles of Db mice. In conclusion, Et.EA extract attenuates insulin resistance by regulating the expression of metabolic enzymes and signaling pathways.

**Keywords:** type 2 diabetes; *Euonymus alatus*; diabetic mice; PI3K/AKT; glycogen synthase

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## 1. Introduction

Type 2 diabetes mellitus (T2DM), a metabolic disease marked by tissue insulin resistance and persistent hyperglycemia, is brought on by obesity, an inappropriate lifestyle, a poor diet, and genetic factors [1,2]. T2DM leads to irregularities in the way the body handles carbohydrates, fats, and proteins, primarily affecting skeletal muscle, body fat, and the liver [3]. The liver regulates glucose homeostasis by controlling glycogen content and influencing lipid metabolism [3,4]. As such, the liver plays a significant role in maintaining glucose levels through insulin signaling, where insulin acts directly by binding insulin receptors on hepatic tissue [5]. There are numerous signaling pathways involved in T2DM, including the PI3K/AKT signaling pathway. After insulin binds to insulin receptor substrate 1 (IRS1), it activates receptor kinase and further phosphorylates PI3K. Upon activation, PI3K forms a complex with the N-terminal PH domain of AKT and further phosphorylates AKT (p-AKT). Protein glycogen synthase kinase 3β (GSK3β) is further phosphorylated into an inactive form, which leads to the dephosphorylation of glycogen synthase. The dephosphorylation of glycogen synthase (GS) thus increases glycogen synthesis [6–8].

To date, numerous antidiabetic medications have been developed for patients, but nearly all of them belong to the category of chemical or biochemical agents, including biguanides and sulphonylureas [9,10]. These medications can only manage blood sugar

levels effectively when taken regularly, and they come with inherent drawbacks, such as the risk of hypoglycemia, lactic acid buildup, and the potential for obesity [11,12]. Given the shortcomings of current medications, plant medicines can be used as an alternative to manage diabetes [13–15]. *Euonymus alatus* (EA) is a medicinal plant belonging to the family Celastraceae with a rich history of traditional use in various Asian countries, notably Korea and China, for addressing a range of health conditions [16–18]. More recently, EA has gained attention, primarily due to research into its bioactive properties related to cancer and diabetes [19–21]. According to previous studies by Park et al., EA extract improves glucose and lipid homeostasis in diabetes. This indicates that EA extract can considerably reduce obesity-related diabetes and non-alcoholic fatty liver disease [20]. EA extract is known for its ability to prevent T2DM-associated obesity through the activation of PPARγ mRNA expression while also effectively reducing hyperlipidemia and insulin resistance [22].

In our earlier research, we found that extracts of *Euonymus alatus* in water and 70% EtOH can exhibit partial antidiabetic effects by decreasing plasma insulin and triglyceride levels [23]. Consequently, the current study explores the preventive properties of EA twig extract against insulin resistance in vivo using a diabetes mouse model, as well as examining the potential mechanisms involved. Additionally, we aim to investigate whether supplementation with EA extract could activate IRS/PI3K and its downstream targets in the diabetes mouse model.

## 2. Materials and Methods

### *2.1. Materials*

The collected twigs of *E. alatus* from Asan, Republic of Korea, in November 2019 were air-dried in the shade and stored at −20 °C for use. The ethanol for the extraction was purchased from Duksan Pure Chemical Co. (Ansan, Republic of Korea). HPLC-grade water, acetonitrile, and trifluoroacetic acid were obtained from Samchun Pure Chemical Co. (Seoul, Republic of Korea), Duksan Pure Chemical Co. (Ansan, Republic of Korea), and Fischer Scientific (Illkirch, France), respectively.

Fetal bovine serum (FBS), penicillin, streptomycin, Roswell Park Memorial Institute (RPMI) 1640), and 0.5% Trypsin-EDTA for cell cultures were obtained from Hyclone (Logan, UT, USA). The antibody IRS-2 (ab134101) was purchased from abcam (Cambridge, MA, USA); p-PI3K (#17366), PI3K (#4257), p-AKT (#9271), AKT (#9272), GSK3 (#12456), p-GSK3 (#5558), p-GS (#3891), and GS (#3886) were obtained from Cell Signaling Technology Inc. (Beverly MA, USA).

### *2.2. Extraction of E. alatus Twigs*

Two different types of solvents were used for the extraction of *E. alatus* twigs. At first, 2 kg of dried twigs were extracted with 70% ethanol (40 L) at 80 °C for 4 h. The extract was filtered through a 1 μm filter, followed by solvent evaporation under reduced pressure to obtain 153.6 g of 70% ethanol extract. Secondly, 2 kg of dried twigs were extracted with deionized water (40 L) at 100 °C for 4 h and filtered through a 1 μm filter, followed by solvent evaporation under reduced pressure to obtain 135.2 g of water extract.

### *2.3. Chromatographic and ESI-MS Detection*

HPLC of both extracts was analyzed at 280 nm by using the Waters Alliance 2695 separation module coupled with a 2998 photodiode array detector and a Sunfire C18 column (4.6 mm × 250 mm, 5 μm). The eluent consisted of 0.08% TFA (A) and acetonitrile (B) in the following multistep linear gradient: 0 min, 5% B; 5 min, 15% B; 40 min, 35% B; 55 min, 50% B; 60 min, 65% B; 70 min, 100% B; 80 min, 100% B. The injection volume was 10 μL with the flow set at 1 mL/min. The DAD spectrum range was set between 200 and 800 nm. The HPLC system was coupled to a Waters micromass ZQ mass spectrometer equipped with an ESI ionization in both positive and negative ion modes. The parameters used were as follows: capillary voltage: 2.50 kV; cone voltage: 40 V; extractor voltage: 3 V; RF lens voltage: 0.3 V; source temperature: 100 °C; desolvation temperature: 200 °C;

desolvation gas flow rate: 350 L/h; cone gas flow rate: 50 L/h; and scanning range: from 100 to 1000 amu.

### *2.4. Animal Experiment and Drug Administration*

Five-week-old male C57BL/ksJ-db/db mice (a type 2 diabetes animal model) ($n$ = 50) and five-week-old male C57BL/6J Jms Slc mice (a normal animal model) were acquired from JoongAng Laboratory Animal Co., Ltd. (Seoul, Republic of Korea). After a one-week acclimatization and adaptation period, the normal and diabetes groups were randomized into the normal group (Nor), diabetes group (Db), metformin group (Met), water extract group (Wa.EA), and 70% EtOH extract group (Et.EA), with each group having 10 mice (Figure 1). The animal breeding room was maintained at a temperature of 20~25 °C, a humidity of 40~50%, a day/night cycle of 12 h, and a sterilized chow diet (Rodent NIH-41M, Zeigler Bros, Gardners, PA, USA), and water was supplied freely. The Nor and Db groups were given tap water orally, whereas the Met group received 200 mg/kg of metmorfin. The Wa.EA and Et.EA were given at a dosage of 500 mg/kg of 70% EtOH extract and water extracts of EA twigs. Water and 70% EtOH extracts of EA twigs were diluted in DW to form a dosage of 500 mg/kg and administered at a volume of 100 μL/mouse every day. They were administered orally for a total of four weeks. During the same period, metformin was given orally at 200 mg/kg. The Nor and Db groups were administered DW (100 μL). Body weight was measured once a week during the experimental period. Animal testing was conducted at Daegu University. The mice were starved for 16 h before sacrifice and administered 2.5% Abertin anesthetic intraperitoneally at 10 μL/g, and blood, liver tissue, and muscle tissue (tensor fasciae latae) were collected. The anesthetic was prepared by dissolving 30 mg of Abertin (2,2,2-Tribromoethanol) (Sigma-Aldrich, Burlington, MA, USA) in 30 μL of TERT amyl alcohol (Sigma-Aldrich) in a 50 °C water bath to prepare a stock solution, and the stock solution was diluted with 37 °C ultra-pure water to a ratio of 1:40 to prepare a 2.5% Abertin working solution. This study was conducted with approval from the Daegu University Animal Experiment Ethics Committee (DUIACC-2020-1-0313-004).

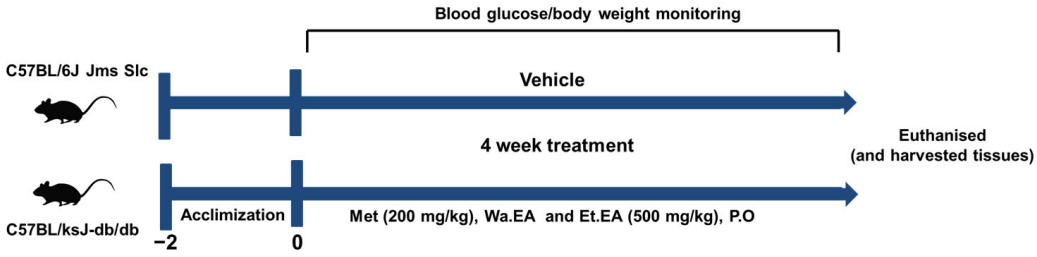



**Figure 1.** Illustration of the experimental design. The experiment was conducted by dividing 50 mice into five groups; the normal group (Nor), the diabetes group (Db), the metformin group (Met), the water extract group (Wa.EA), and the 70% EtOH extract group (Et.EA), ($n$ = 10). The Nor and Db groups were administered tap water, while the Met group received a dosage of 200 mg/kg of metmorfin. The Wa.EA and Et.EA groups received a dosage of 500 mg/kg of 70% EtOH extract and water extract of *Euonymus alatus* twig extracts. Other experiments were performed after dissection.

### *2.5. Glycogen Synthase Activity*

GS activity in the liver or muscle tissue (tensor fasciae latae) was measured by using RIPA buffer (GenDEPOT, #03202015) at 100 mg/mL using a glycogen synthase microplate assay kit (MyBiosource, #MBS8309693, San Diego, CA, USA) following the manufacturer's instructions. Absorbance at 340 nm was measured with a microplate reader (Tecan, Männedorf, Switzerland) after quantitation with BSA. The calculation formula is as follows:

$$Glycogen\ sythase\left(\frac{\mathrm{U}}{\mathrm{mg}}\right) = 400\frac{\mathrm{nM}}{\mathrm{mL}} \times 0.2\ \mathrm{mL} \times \frac{\frac{OD.\ Sample\ 10s\ -\ OD.\ Sample\ 70s}{OD.\ Standard\ -\ OD.\ Blank}}{\frac{(sample\ volume \times Protein\ concetation)}{Time}}$$

### *2.6. Glycogen Determination*

Glycogen determination was performed with reference to the phenol sulfate method [24]. First, 30% KOH was added at 1 mg/mL to homogenize the tissues. After that, tissue lysates were mixed with ethanol and incubated for 2 h at room temperature, and glycogen was separated by centrifugation. Glycogen samples were treated with DW, 5% phenol, and sulfuric acid. Absorbance at 490 nm was measured using a microplate reader (Tecan, Männedorf, Switzerland).

### *2.7. Western Blot*

Murine liver and muscle samples were weighed and homogenized in ice-cold RIPA lysis buffer (0.15 M sodium chloride, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 50 mM Tris adjusted to pH 7.5) supplemented with a protease inhibitor cocktail (GenDEPOT, Barker, TX, USA) using a tissue homogenizer. Tissue lysates were centrifuged at 12,000× *g* for 15 min, and protein quantitation was performed with a Bio-Rad protein assay reagent. Proteins were subjected to SDS-PAGE electrophoresis and then transferred onto a PVDF membrane. The membranes were immersed in PBS-Tween-20 (PBS-T) buffer for 1 h and then incubated with the required primary antibodies in PBS-T with 5% (*w*/*v*) skimmed milk overnight at 4 °C. Then, the membranes were washed three times with PBS-T and incubated with HRP-conjugated secondary antibodies at room temperature. Blots were prepared by using an enhanced chemiluminescence (ECL) detection reagent (Pierce Biotechnology, Rockford, IL, USA) and imaged in a luminescent image analyzer (ImageQuant Las 500, Cytiva, Tokyo, Japan). Antibodies against target proteins and their relevant information are listed in 2.1.Materials.

### *2.8. Statistical Analysis*

The statistical significance of the results obtained from the experiment was evaluated using SPSS (Statistical Package for the Social Sciences, Version 25.0, IBM Corp., Armonk, NY, USA). The experimental data are presented as mean ± standard deviation (SD), and the statistical significance among each group was post-tested using the Duncan method through multiple comparisons. A $p$-value of <0.05 was considered statistically significant.

## 3. Results

### *3.1. HPLC Analysis for EA Twig Extracts*

Different types of flavonoids, such as catechin (peak 1), epicatechin (peak 2), taxifolin (peak 3), aromadendrin (peak 4), and naringenin (peak 5), were identified, as shown in Figure 2A. The HPLC chromatogram suggested that catechin was the major constituent of the EA twig extract. In contrast, the water extract of EA twigs showed the presence of only catechin and epicatechin, represented as peak 1 and 2, respectively (Figure 2B).

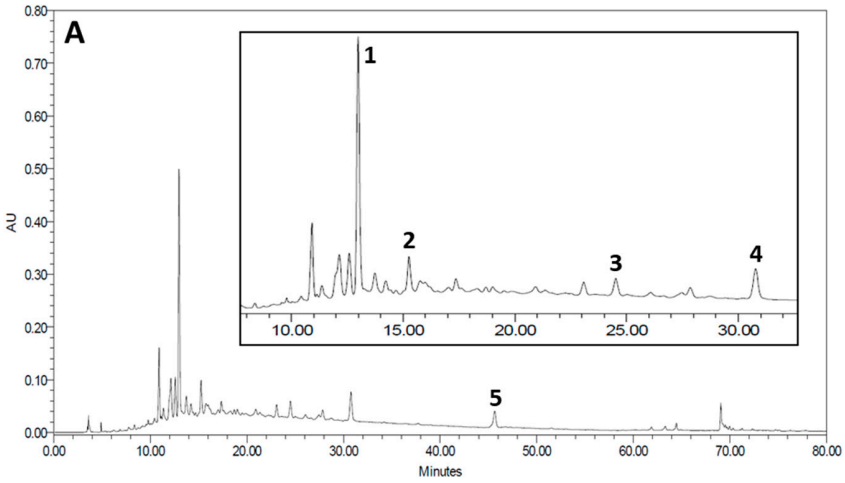



**Figure 2.** *Cont.*

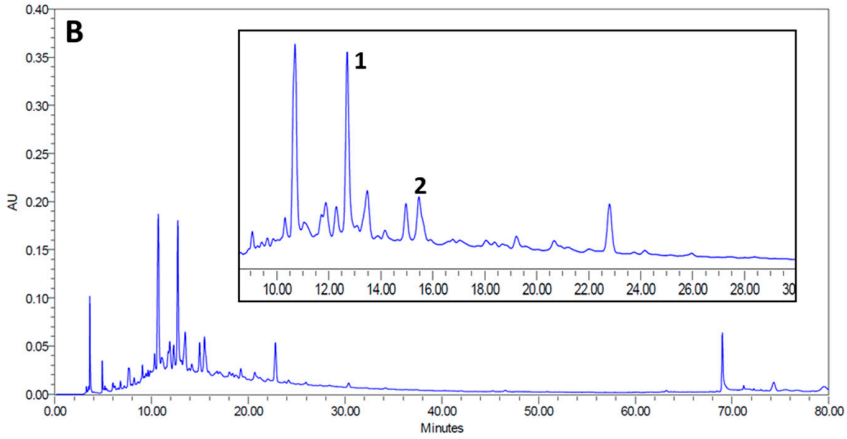



**Figure 2.** HPLC chromatogram of EA twig extract at 280 nm. (**A**) The 70% ethanol extract, showing identified constituents, namely catechin (peak 1), epicatechin (peak 2), taxifolin (peak 3), aromadendrin (peak 4), and naringenin (peak 5). (**B**) The water extract, possessing only catechin (peak 1) and epicatechin (peak 2) among the identified flavonoids.

### *3.2. Effect of EA Extract on Body Weight*

From the beginning of the study, we measured the body weight [23]. During the initial period of the study, there was an increase in the body weight of the C57BL/ksJ-db/db mice compared to the Nor group, but there was no difference between the drug-treated diabetes model groups (Figure 3). At the conclusion of the study, there was no difference in body weight changes between the pharmacological intervention (Met, Wa.EA, and Et.EA) and db groups.

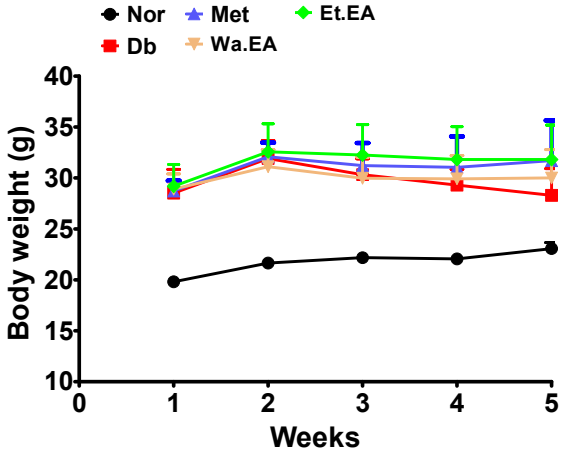



**Figure 3.** Body weight changes in experimental groups. Body weight of treated and untreated Db mice. Nor: normal group; Db: diabetes group; Met: metformin group; Wa.EA: water EA extract group; Et.EA group: 70% ethyl alcohol EA extract group. Data are represented as mean ± S.D ($n$ = 10/group).

### *3.3. Effect of Oral Administration of Extract on Glycogen Content in Different Tissues*

Liver glycogen stores energy and plays an important role in blood glucose homeostasis. After 4 weeks of oral administration of the EA extract to the type 2 diabetes model (Db mouse), the glycogen levels in the liver and muscle were compared to those of the normal mice ($p < 0.01$, $n = 7$). The study revealed that the Db mice treated with Et.EA had considerably higher liver glycogen levels than the Db mice treated with Wa.EA or metformin (Figure 4A). The muscle glycogen levels were significantly lower in the Db mice compared to the Nor mice. When compared to the Db group of mice, the lowered muscle glycogen was greatly restored by the Et.EA and Wa.EA treatments, but to a lesser extent by the met treatment.

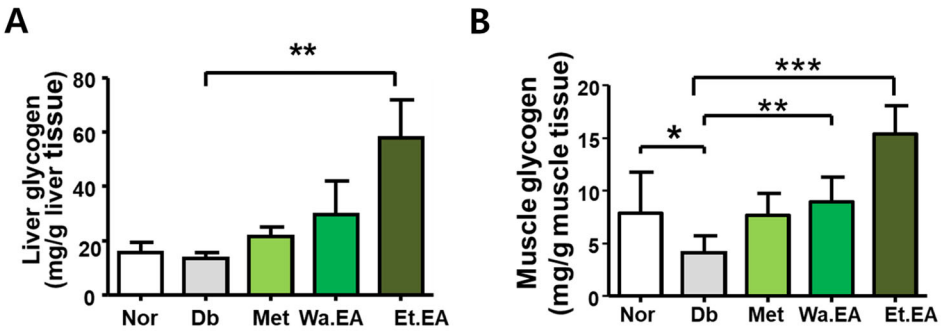



**Figure 4.** EA extract induces glycogen synthesis in Db mice. Nor: C57BL/6J Jms Slc mice treated with DW. Db: C57BL/6J Jms Slc mice treated with DW. Met: Db mice treated with 200 mg/kg/day of metformin. Wa.EA: Db mice treated with 500 mg/kg/day of water EA extract. Et.EA: Db mice treated with 500 mg/kg/day of 70% EtOH EA extract. (**A**) Liver and (**B**) muscle glycogen content for different groups of mice. The data are presented as the mean ± SD of three experiments ($n$ = 10/group, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

### *3.4. Effect of Oral Administration of EA Extract on the Liver Insulin PI3K–AKT–GSK3β Signaling Pathway*

We tested whether the EA twig extract impacts the PI3K–AKT–GSK3β insulin signaling pathway in the Db mice using a Western blot assay. The IRS2 protein expression in the Db mice decreased in comparison to the normal mice, as Figure 5 illustrates, but it increased significantly when the mice were treated with the EA extract (70% EtOH). Comparing the Wa.EA and Met therapies to the Db group revealed no changes. Furthermore, the Db mice had a lower phosphorylation of PI3K than the normal mice. This lowered the phosphorylation of PI3K, which was increased significantly by Et.EA treatments. Phosphorylated AKT and GSK3β exhibited an elevated pattern in the Db mice in comparison to the normal mice, while treatment with Et.EA further enhanced their expression. While the phosphorylation of GS increased in the Db control mice, it was decreased significantly by the Et.EA treatment.

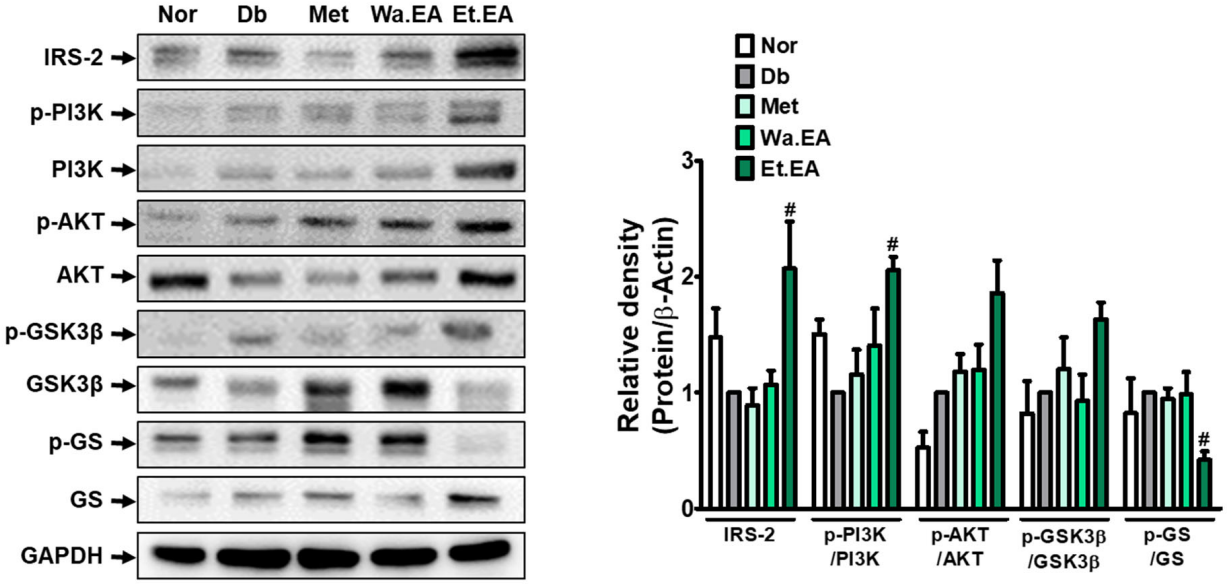



**Figure 5.** EtOH extract induces IRS-2/PI3K/AKT/GSK3β/GS expression in the liver of Db mice. Nor: C57BL/6J Jms Slc mice treated with DW. Db: C57BL/6J Jms Slc mice treated with DW. Metformin: Db mice treated with metformin. Wa.EA: Db mice treated with water EA extract. Et.EA: Db mice treated with 70% EtOH EA extract. The data are presented as the mean ± SD of triplicate samples. # $p < 0.05$ compared to the Db group of mice.

### *3.5. Effect of Oral Administration of EA Extract on Muscle PI3K–AKT–GSK3β Expression*

We also investigated how EA extract affected the PI3K–AKT–GSK3β signaling pathway in muscle. As seen in Figure 6, the expression of insulin signaling molecules, such as IRS-1, showed little change in the Db mice in comparison to the normal mice; however, it was increased by the EA extract (Water or EtOH) and metformin. Treatment with the EA extract (water or EtOH) slightly increased the phosphorylated PI3K, while it highly increased the phosphorylated form of AKT and GSK3β expression. The expression of phosphorylated GS was decreased significantly by the EA extract (water or EtOH), while the met treatment showed no changes compared to the vehicle-treated Db mice. The results demonstrate that the EA extract (EtOH) was effective in enhancing PI3K–AKT–GSK3β expression in muscles.

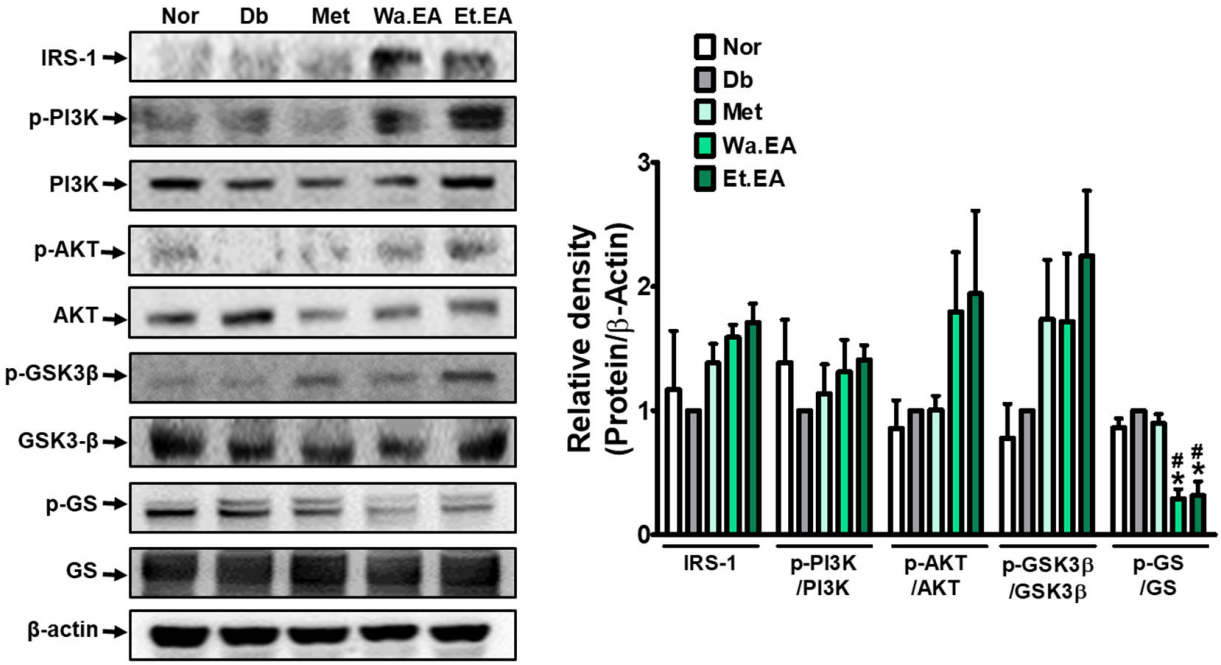



**Figure 6.** Expression of IRS-2/PI3K/AKT/GSK3β/GS in muscles of Db mice. Nor: C57BL/6J Jms Slc mice treated with DW. Db: C57BL/6J Jms Slc mice treated with DW. Metformin: Db mice treated with metformin. Wa.EA: Db mice treated with water EA extract. Et.EA: Db mice treated with 70% EtOH EA extract. The data are presented as the mean ± SD of triplicate samples. * $p < 0.05$ compared to the normal group of mice. # $p < 0.05$ compared to the Db group of mice.

### *3.6. Effect of Oral Administration of Extract on Glycogen Synthase (GS) Activity in the Liver and Muscles*

Insulin levels play a crucial role in regulating glycogen synthesis, which is essential for maintaining blood glucose levels. Glycogen synthase (GS) is the rate-limiting enzyme during glycogen synthesis [25,26]. As shown in Figure 7A, the activity of GS in the liver of the Db mice was observed to be less than in the Nor mice, indicating less glycogenesis. However, the lowered GS activity in the Db mice was significantly restored in the Et.EA-treated Db mice. In the Met-treated Db mice, the GS activity was less restored, while the Wa.EA-treated mice showed no changes. We observed that the muscles' GS activity in the EA extract-treated Db mice showed a similar pattern as in the liver tissues (Figure 7B).

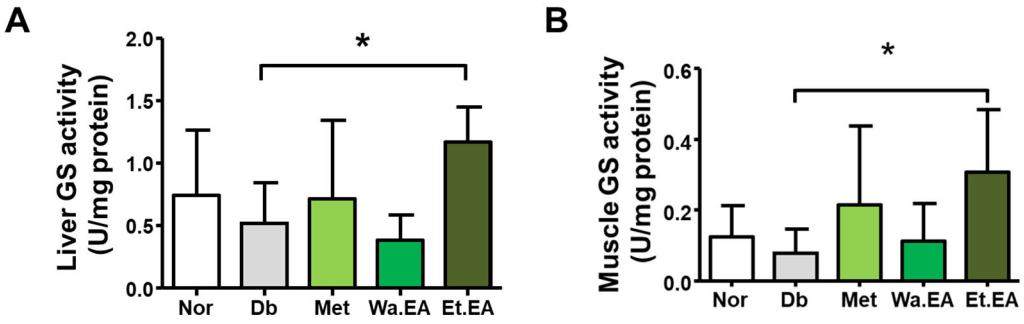



**Figure 7.** EtOH extract induces glycogen synthase activity in the liver and muscle of Db mice. Nor: C57BL/6J Jms Slc mice treated with DW. Db: C57BL/6J Jms Slc mice treated with DW. Met: Db mice treated with 200 mg/kg/day of metformin. Wa.EA: Db mice treated with 500 mg/kg/day of water EA extract. Et.EA: Db mice treated with 500 mg/kg/day of 70% EtOH EA extract. Glycogen synthase activity in (**A**) liver and (**B**) muscle for different groups of mice. The data are presented as the mean ± SD of three experiments ($n$ = 10/group, * $p < 0.05$).

## 4. Discussion

Without producing any negative effects, the perfect antidiabetic medication would help diabetic patients with insulin resistance and glucose metabolism [27]. Nonetheless, the prospect of discovering novel phytochemical-based antidiabetic medications can be alluring since these compounds possess anti-hyperglycemic and safe properties. In our research, we employed the C57BL/KsJ-db/db mouse model, which is a well-known model of obesity-induced type 2 diabetes. C57BL/KsJ-db/db mice have mutations in the leptin receptor gene that cause obesity and alter pituitary functioning [28,29]. Hence, in the present study, using a type 2 diabetic mouse model, for the first time we demonstrated that EA twig (70% EtOH) extract has superior benefits than metformin and Wa.EA extract in improving insulin resistance. In the Db mice, the EA extract (70% EtOH) increased glycogen synthesis through the enhancement of the PI3K/AKT/GSK3β pathway.

Our results showed that EA extract caused no improvement in body weight. Similarly, our previous study found that the mice in the EA treatment group had lower serum triglyceride (TG) levels with no change in the serum total cholesterol (TC) levels [23]. Excess glucose is metabolized to acetyl-CoA, which then undergoes TG synthesis through de novo lipogenesis [30]. Insulin controls the transcription of factors such as Sterol regulatory element-binding proteins-1c (SREBP-1c) and carbohydrate-responsive element-binding protein (ChREBP), and they are involved in the induction of lipogenic genes [31]. In addition, our earlier study also showed a downregulation of insulin levels by EA twig extract that may, at least in part, be responsible for the improvement in dyslipidemia in the db/db mice. Previous studies have shown that twig EA extract has an effect on fasting blood glucose in T2DM rats [16]. The EA extract showed no effect on blood glucose in the Db mice. Similarly, when Et.EA extract was administered to Db mice, it resulted in a modest decrease in plasma insulin levels. However, the Met- and Et.EA-treated mice had reduced water consumption when compared to the untreated controls, with a pronounced effect evident in the last week of treatment [23]. Research has demonstrated that leptin levels are insignificantly low in patients with diabetes. These results might be due to the presence of defective leptin during a diabetic condition [32,33]. The administration of recombinant leptin, either centrally or peripherally, was found to have no effect on food intake or body weight in the db/db mice [34,35]. Significantly, the long-term administration of the EA extract can give rise to significant results in diabetic mice.

The primary modulator of glycogenesis in the liver and muscles is insulin [4]. Others have previously noted that diabetic rats had lower glycogen levels in their skeletal and liver muscles [36,37]. Similarly, in our study, both skeletal muscle and hepatic glycogen decreased in the Db mice, which might be due to the inactivation of the glycogen synthase system. When a 70% ethanol extract of EA was given to diabetic rats for four weeks, the levels of glycogen in the muscles and liver increased significantly and in a graduated man-

ner. Therefore, this study focuses on the extract's potential antidiabetogenic effect, which could be enhanced by improving the liver and muscle glycogenesis processes. The liver and skeletal muscle have vital roles in preserving the equilibrium of energy metabolism by controlling blood glucose and lipid levels through glycogenolysis, or gluconeogenesis. A surge in glucose production and a fall in glycogen lead to the metabolic dysfunction evident in T2DM. Consequently, it is advantageous to treat T2DM by inhibiting glucose synthesis and promoting glycogen synthesis [38]. In the present study, we studied the regulatory role of EA twig extract in the protein expression of enzymes linked to glucose metabolism, such as PI3K/AKT, and GS in Db mouse liver and muscle. Pre-experiments revealed that even when exposed to the highest concentration of 50 μg/mL, the islet β (INS1) cells treated with the extract derived from EA (water and 70% ethanol) twigs exhibited less than 50% cell mortality [23]. The relatively low level of toxicity exhibited by the EA twig extracts raises the prospect that they could be potentially safe for users.

EA has gained recognition as a useful treatment and preventative measure for a number of illnesses in recent years, as it is composed of total alkaloids, saponins, flavonoids, and polysaccharides [16]. Furthermore, an *E. alatus* formula along with diet and exercise in patients with impaired glucose tolerance was found to lower blood glucose compared to that of untreated patients [16]. Previous research has demonstrated that EA therapy effectively reduced the impact of lipid-induced diabetes and obesity in ICR mice subjected to a high-fat diet while not inducing any notable toxic effects [20]. The active components of *E. alatus*, such as kaempferol and quercetin, enhanced insulin-mediated glucose absorption in mature 3T3-L1 cells [39]. Furthermore, it has been demonstrated that rutin, one of the components of *E. alatus*, lowers glucose and fat plasma levels while raising PPARγ expression in Db mice [40]. In our study, catechin, epicatechin, aromadendrin, taxifolin, and naringenin were identified as the main active compounds of the 70% EtOH EA twig extract. However, only catechin and epicatechin were identified in the Wa.EA twig extract. In terms of antidiabetic effectiveness and component identification, the result revealed that the EtOH EA extract exhibited better antidiabetic activity than the water EA extract, which can be attributed to the presence of the aforementioned phytochemicals. Similarly, numerous compounds from the *E. alatus* extracts, including aromadendrin, epifriedelanol, protocatechuic acid, β-sitosterol, and quercetin, were also reported to have hyperglycemic activity and be protective against experimental diabetic nephropathy [41–44]. In addition, another study showed that epicatechin possesses insulin-like action and can stimulate insulin release from isolated rat islets [45]. Apart from flavonoids, some triterpenes from EA twigs have also been shown to have potent α-glucosidase inhibitory effects [46].

The most crucial approach to the management and prevention of type 2 diabetes is to alleviate insulin resistance [32]. Therefore, we unraveled the mechanism of action of EA extract by examining the insulin signaling cascade. Our result showed that there was a significant increase in IRS2 and p-PI3K but a significant decrease in p-GS protein expression in the liver of the Db mice. Nevertheless, the EA extract treatment also increased the expression of p-AKT in the liver of the Db mice compared to the untreated Db mice. AKT, a key mediator in the activation of the biochemical mechanism in glucose metabolism, plays a crucial role as one of the primary effectors within the PI3K cascade pathway [47]. Additionally, the treatment with EA extract also increased the expression of GSK3β, a downstream component of AKT, in comparison to the Db mice. GSK3β, an important enzyme, is regulated by phosphorylated AKT. GSK3β is a crucial negative regulator of the insulin signaling pathway that negatively controls insulin secretion, glucose homeostasis, and glucose transport, in addition to gluconeogenesis and glycogen formation [48,49]. GS plays a direct role in the metabolism of glycogen, boosting the body's production of glycogen and reducing blood glucose levels [50]. The study also found that the Et.EA treatment also increased IRS1 expression in the muscles of the Db mice. Moreover, coinciding with the IRS-1 increase, the Et.EA extract also elevated p-PI3K, p-AKT, and p-GSK3β and reduced p-GS expression in the Db mice. Therefore, the Et.EA extract dramatically elevated the

expression of signaling molecules regulating the PI3K/AKT pathway, suggesting that it has a favorable impact on muscle–liver insulin transduction.

## 5. Conclusions

Overall, our research reveals that the brief administration of EA twig extract to db/db mice enhances glycogen synthase activity and accelerates glycogen synthesis in both the liver and muscle. Thus, EA twig extract can reduce insulin resistance by activating the PI3K/AKT pathway and decreasing the p-GS pathway. Notably, in terms of the overall antidiabetic effects, the Et.EA extract outperformed metformin, and the Wa.EA extract improved insulin resistance with relatively fewer side effects. However, there are still a few crucial issues to take into account. To ascertain the safety and effectiveness of EA extract as a supplement that lowers blood sugar, more thorough studies with longer intervention periods are necessary, and additional research on the mechanism of action is warranted. Considering its effectiveness and lack of toxicity, it appears imperative to use EA twig extract as an antidiabetic agent in pharmaceuticals and functional foods.

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