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Flavonoids from green tea reverse insulin resistance and inflammation: *in vitro* and *in vivo* evidence

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ABSTRACT

Flavonoids are a class of polyphenolic compounds that can be isolated from green tea (*Camellia sinensis*). This study has investigated the *in vitro* and *in vivo* effects of green tea flavonoids on insulin resistance and inflammation. Flavonoids were extracted from green tea using ethanol extraction followed by purification through the employment of HPLC. *In vitro* experiments were conducted on two cell lines in order to assess glucose uptake and inflammatory marker expression, while *in vivo* studies involved streptozotocin-induced diabetic mice treated with green tea-derived flavonoids (240 mg/kg) for 30 days, and with their blood glucose and glycated haemoglobin (HbA1c) levels being analysed. Our results revealed that the flavonoid treatment could significantly increase glucose uptake *in vitro* ($p<0.05$), while diabetic mice exhibited lower fasting blood glucose ($p<0.001$) and HbA1c ($p<0.01$) levels compared to untreated controls. In conclusion, these findings suggest that flavonoids may serve as effective adjunct therapies for diabetes and inflammation.

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1. Introduction

Diabetes mellitus is a complex metabolic condition shaped through hereditary genetics, environmental influences, and lifestyle choices. It is considered as a primary contributor to serious problems such as blindness, delayed wound healing, erectile dysfunction, kidney failure, and cardiovascular diseases, leading to extensive or-

PHARMAKEFTIKI, 37, 2S, 2025 | 67-71

gan damage and compromised cellular processes. It is a chronic condition marked by sustained hyperglycaemia, primarily resulting from defects in insulin secretion, insulin action, or a combination of both. Two primary forms for diabetes exist: type 1 (defined through the autoimmune destruction of pancreatic β-cells resulting to complete insulin deficiency that necessitates continuous exogenous insulin injection) and type 2 (which represents 90% of global cases of diabetes and is characterized by insulin resistance and relative insulin deficiency that needs to be controlled through lifestyle adjustment)[1].

Flavonoids, a variety of polyphenolic substances prevalent in plants, constitute an important class for phytochemicals with possible antidiabetic effects. These chemicals, comprising flavones, flavonols, anthocyanins, and isoflavones, demonstrate multifaceted functions that affect inflammation and metabolic signalling pathways[2,3]. Flavonoids serve like powerful antioxidants and have been shown to exert anti-inflammatory, anticancer, antiviral, and cardioprotective effects[4]. Additionally, they are essential for the regulation of glucose and lipid metabolism, mitigating dyslipidaemia and insulin resistance, and altering stress-responsive signalling pathways[5]. Flavonoids also possess considerable potential as nutraceuticals and have been shown to exert their anti-diabetic effects through many pathways, such as the modulation for glucose metabolism, the attenuation for oxidative stress, and the enhancement of insulin sensitivity[2–5].

The aim of is study was to investigate the *in vitro* and *in vivo* effects of green tea flavonoids on insulin resistance and inflammation.

2. Methodology

2.1. Flavonoid extraction and purification

Flavonoids were extracted from green tea (*Camellia sinensis*) using ethanol-water solvent extraction at high temperature (100°C) under controlled laboratory conditions. The extract was filtered, concentrated at 100°C, and lyophilized. HPLC was used in order to purify the extracted flavonoids, ensuring a purity level above 95%.

2.2. *In vitro* experiments

To evaluate the effect of flavonoids on glucose metabolism, two cell lines were used: the INS-1E cell line (which is a rat insulinoma cell line that maintains notable differentiation to a stable β-cell phenotype achieved by selection for high insulin secretion) and the C2C12 cell line (which is an immortalized mouse myoblast cell line). Cells were treated with flavonoids at concentrations of 10, 50, and 100 μM, and glucose uptake was measured using a glucose uptake assay method. The levels of inflammatory cytokines such as internleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α) were measured by ELISA. Mechanistic analyses involving Western blotting were used in order to assess the AMP-activated protein kinase (AMPK) activation, the glucose transporter type 4 (GLUT4) translocation, and the nuclear factor erythroid 2-related factor 2 (Nrf2) signalling.

2.3. *In vivo* experiments

The *in vivo* experiments of our study were approved by Kufa University (protocol number: 116; date: 20-Mar-2023). In brief, male BALB/c mice (weighting 30 g) were obtained from the market and were housed at suitable conditions (temperature: 25°C; 12:12 h light : dark cycle; free food and water access). For the induction of diabetes, mice were injected intraperitoneally with streptozotocin (at 240mg/kg). Mice were then randomly allocated into two groups (n=5 per group): (i) the control group (untreated diabetic mice) and (ii) the flavonoid-treated group (receiving flavonoids intraperitoneally at 240 mg/kg/day, for 30 days). Blood samples were collected through the heart on day 30 and the therapeutic effects of the flavonoids were evaluated through the measurement of fasting blood glucose, glycated haemoglobin (HbA1c), and low-density lipoprotein (LDL) cholesterol.

PHARMAKEFTIKI, 37, 2S, 2025 | 67-71

Table 1. The effect of a 30-day administration of green tea-derived flavonoids (240 mg/kg/day) on the levels of fasting blood sugar, glycated haemoglobin (HbA1c), and low-density lipoprotein (LDL) cholesterol of streptozotocin-induced diabetic mice.

Parameter	**Control group** (mean ± SD)	**Flavonoid-treated group** (mean ± SD)	***p*-value**
Fasting blood glucose (mg/dL)	220 ± 15	135 ± 10	<0.001
HbA1c (%)	8.5 ± 0.5	6.2 ± 0.4	<0.01
LDL cholesterol (mg/dL)	130 ± 8	90 ± 6	<0.05

2.4. Statistical analysis

The statistical analysis was performed by using the SPSS software (version 25.0). One-way ANOVA and Tukey's *post hoc* test were used in order to evaluate the effects of the flavonoid treatments; differences with a *p*-value below 0.05 were deemed as statistically significant.

3. Results and Discussion

Green tea-derived flavonoid administration markedly decreased fasting blood glucose ($p<0.001$) and HbA1c ($p<0.01$) levels in diabetic mice as compared to those of the control group (Table 1). On the other hand, the exposure to green tea-derived flavonoids (15 μM; 24 h) increased insulin secretion, insulin mRNA levels, and GLUT2 expression in INS-1E cells. An activation of the insulin signalling pathway was also observed in these cells, including an increased phosphorylation of IRS-1, PI3Kp85, and AKT, as well as an increased association between PI3K and IRS-1 levels. In the C2C12 cells, the exposure to green tea-derived flavonoids (40 μM; 1 h) led to increased glucose uptake and GLUT4 translocation. Moreover, a significant phosphorylation of AMPKα and of ACC was observed, indicating an AMPK pathway activation. Co-treatment with insulin further enhanced p-AMPKα, p-ACC, and p-Akt protein levels.

Our findings indicate, that green tea-derived flavonoids can reduce blood glucose levels *in vivo*, and exert insulin-sensitizing activities *in vitro*, thereby qualifying them as a potential alternative or adjunct option to traditional antidiabetic treatments[6]. The treatment with flavonoids resulted in a significant decrease in the fasting blood glucose levels of diabetic mice. This discovery confirms the capacity of flavonoids to stimulate essential metabolic pathways, including the promotion of GLUT4 translocation and the AMPK activation through substances such as epicatechin, consequently enhancing glucose uptake within skeletal muscles. The latter is known to enhance lipid metabolism and glucose absorption, directly targeting insulin resistance; a characteristic of type 2 diabetes. Catechins and epigallocatechin gallate (EGCG) have been found to augment glucose transport independently of insulin, while the inhibition of the vascular endothelial growth factor by flavonoids such as EGCG has exhibited potential in mitigating problems such like diabetic retinopathy through the obstruction of pathological neovascularization[7].

The flavonoids' capacity to block enzymes such like α-glucosidase and α-amylase diminishes glucose absorption from the gastrointestinal tract, consequently effectively thought to regulate postprandial hyperglycaemia. In fact, flavonoids have shown similar efficiency to current antidiabetic therapies in regulating critical indicators, including fasting blood glucose levels, HbA1c levels, and lipid profiles. Quercetin and rutin have been shown to safeguard against diabetic nephropathy through the diminishing of advanced glycation end products and the enhancement of renal function indicators[8]. Moreover, the natural provenance for flavonoids and their negligible adverse effects qualify them as

PHARMAKEFTIKI, 37, 2S, 2025 | 67-71

a safer option to synthetic pharmaceuticals. However, the bioavailability of flavonoids presents a difficulty that necessitates the development of improved formulations[9].

Notwithstanding the encouraging findings, this study possesses specific limitations. The bioavailability of flavonoids significantly varies based on their chemical structure and the host organism's metabolic processes. Future research should concentrate on optimizing flavonoid delivery modalities, including the use of nano-formulations or their co-administration alongside bioenhancers. Furthermore, one should note that although our *in vivo* and *in vitro* results turn out to be suggestive of the anti-diabetic potential of green tea-derived flavonoids, clinical trials are crucial in proving that these findings could be safe and useful for humans. Research must investigate the long-term effects of flavonoid supplementation and its combination with other drugs in order to guarantee safety and efficacy[10].

4. Conclusion

Our findings reveal that the flavonoid treatment could significantly increase glucose uptake *in vitro*, while diabetic mice exhibited lower fasting blood glucose and HbA1c levels compared to untreated controls. Our findings also indicate that green tea-derived flavonoids possess considerable potential as therapeutic agents for the management of diabetes and its complications. Flavonoids can improve glycaemic control through the targeting of critical associated molecular pathways, including the AMPK activation and the GLUT4 translocation; molecular steps that are pivotal for the pathogenesis of diabetes mellitus.

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Conflicts of interest

None exist.

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PHARMAKEFTIKI, 37, 2S, 2025 | 67-71

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