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Effects of plant flavonoids and selenium on pancreatic hormones and glucose homeostasis in autoimmune thyroiditis

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Abstract : Autoimmune thyroiditis (AIT) is a systemic autoimmune disorder that extends beyond thyroid dysfunction and is associated with disturbances in pancreatic endocrine secretion and glucose metabolism. The present study aimed to evaluate the effects of selected plant flavonoids—dihydroquercetin, chrysoeriol, luteolin, resveratrol, kaempferol, and thameflaside—administered alone or in combination with selenium on pancreatic hormone secretion, glycemic control, and hepatic glycogen content in an experimental rat model of AIT. AIT was induced in male Wistar rats using thyroglobulin and Freund's adjuvant. After confirmation of autoimmune thyroiditis, animals received flavonoid treatment (20 mg/kg body weight), with or without selenium, for 14 days. Serum concentrations of insulin, glucagon, amylin, and somatostatin were determined by immunoassay. Glycated hemoglobin (HbA1c) was measured as an indicator of long-term glycemic control, and hepatic glycogen content was assessed in liver homogenates. AIT induction resulted in marked metabolic dysregulation characterized by reduced insulin and amylin secretion, elevated glucagon levels, impaired somatostatin regulation, increased HbA1c, and depletion of hepatic glycogen stores. Flavonoid treatment produced compound-specific corrective effects. Luteolin and kaempferol, particularly in combination with selenium, showed the strongest efficacy by restoring pancreatic hormone balance, reducing hyperglucagonemia, improving HbA1c levels, and replenishing hepatic glycogen toward physiological ranges. Dihydroquercetin and chrysoeriol exerted moderate protective effects, whereas resveratrol and thameflaside showed limited efficacy. In conclusion, autoimmune thyroiditis induces significant disturbances in pancreatic endocrine function and hepatic glucose metabolism, while flavonoid–selenium combinations may represent promising strategies for correcting AIT-associated metabolic dysfunctions.

Key words: amylin, autoimmune thyroiditis, glucagon, insulin, somatostatin

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Introduction

Autoimmune thyroiditis (AIT) is a chronic inflammatory disease of the thyroid gland characterized not only by disrupted synthesis of thyroid hormones but also by profound disturbances in systemic metabolic processes [1]. accumulating evidence over recent years indicates that AIT should not be regarded solely as a localized endocrine disorder. moreover, it is associated with dysfunctional changes affecting multiple organs, including the pancreas and liver [2]. in particular, disturbances in pancreatic endocrine secretion and dysregulation of glucose homeostasis have been identified as vital metabolic consequences related to AIT [3, 4].

The endocrine pancreas secretes several hormones—insulin, glucagon, somatostatin, and amylin—that play central roles in the regulation of glucose metabolism and overall energy balance. Under autoimmune and inflammatory conditions, dysregulation of pancreatic hormone secretion may occur, which triggers hyperglycemia, disturbed glucose tolerance, and deterioration of long-term glycemic control [5]. These changes are commonly accompanied by increased glycated hemoglobin (hba1c) levels and depletion of hepatic glycogen stores, reflecting the progression of systemic metabolic dysfunction [6].

oxidative stress, mitochondrial dysfunction, and increased production of proinflammatory mediators observed in AIT have been shown to adversely affect the functional integrity of pancreatic β -, α -, and δ -cells. consequently, insulin secretion is reduced, glucagon secretion is relatively increased, and the regulatory role of somatostatin becomes disrupted [7, 8]. This imbalance in pancreatic hormone signaling may lead to suppression of hepatic glycogen synthesis and reduction of glucose uptake by peripheral tissues.

In recent years, plant-derived flavonoids have attracted growing interest as biologically active com-

pounds with the potential to regulate metabolic and endocrine disorders [9,10]. Flavonoids are typically existed in plants as glycosides and require enzymatic hydrolysis for absorption in their aglycone form. Deglycosylation of flavonoid conjugates occurs in the small and large intestine and is primarily mediated by enzyme lactase [11]. Flavonoids such as dihydroquercetin, (taxifolin) (DhQ), chrysoeriol (c hry), Luteolin (Lt), resveratrol (RSV), kaempferol (KMF), and thamiflaside (Tm) exhibit potent antioxidant, anti-inflammatory, and cytoprotective properties, enabling them to protect hormone that produces pancreatic cells and restore their functional activity [12–17]. However, their effects on pancreatic hormone secretion under the AIT condition remain unclear [18].

Selenium (Se) is an essential component of the antioxidant defense system, supporting the activity of glutathione peroxidase and other selenoproteins, and plays a key role in the peripheral metabolism of thyroid hormones [19]. The combined administration of flavonoids and Se may enhance antioxidant capacity and exert complementary metabolic effects [20]. Thus, determining the impact of flavonoid–Se combinations on endocrine function of pancreas and hepatic glycogen metabolism under conditions of AIT is scientifically justified relevance.

The chemical structures of the studied flavonoids (DHQ, Chry, Lt, RSV, KMF, and Tm) were obtained from previously published sources [21–26]. DhQ, chry, Lt, RSV, KMF, and Tm are plant-derived polyphenolic compounds with pronounced antioxidant and anti-inflammatory functions (Fig. 1) [27–29]. experimental studies note that these substances modulate key molecular pathways involved in autoimmune and metabolic disorders, including suppression of nuclear factor kappa B (NF- κ B)–mediated inflammatory signaling, reduction of pro-inflammatory cytokine production, and mitigation of oxidative stress [30]. For example, Lt has

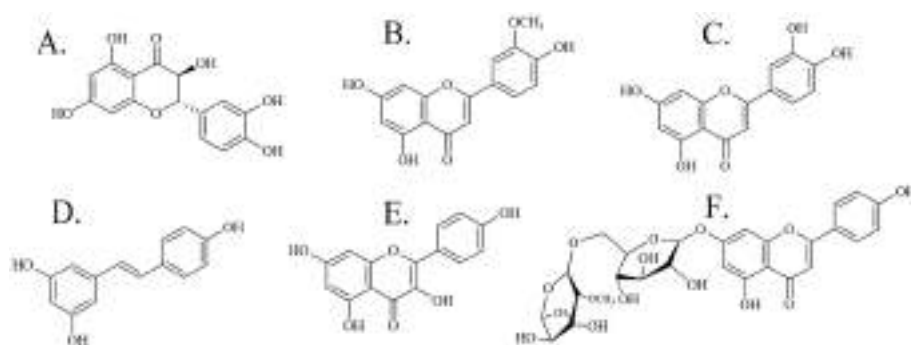


Fig. 1. Chemical structure of flavonoids: A-DHQ [21], B-chry [22], c-Lt [23], D-rSv [24], E-KmF [25], F-Tm [26].

been shown to inhibit NF- κ B and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways and to restore the balance between regulator T (Treg) cells and T helper 17 (Th17) [31].

collectively, these bioactive compounds exhibit cytoprotective effects on pancreatic β -cells, support insulin secretion, and contribute to the maintenance of glucose homeostasis, providing a mechanistic basis for their potential use in correcting pancreatic endocrine dysfunction associated with AIT.

Aim of the study

The aim of the present study was to comprehensively evaluate the effects of various flavonoids, both alone and in combination with Se, on pancreatic endocrine secretion, glycemic control parameters under conditions of experimental AIT.

Materials and Methods

Experimental animals and housing conditions

adult male Wistar rats (weighing 180–220 g, 8–10 weeks old) were used in this study. The animals were obtained from a certified laboratory animal breeding facility and were acclimatized for one week prior to the experiment. rats were housed in standard polypropylene cages (5 animals per cage) under controlled environmental conditions: temperature $22 \pm 2^\circ\text{C}$, relative humidity 55–65%, and a 12 h light/12 h dark cycle. Animals had free access to standard laboratory chow and tap water *ad libitum*.

all procedures were performed in compliance with the European Directive 2010/63/EU for animal experiments and were approved by the institutional animal care and ethics committee of the national university of Uzbekistan (Ethical approval number: NUU-AE-2024-11/27).

Induction of experimental AIT

AIT was induced based on the protocol described by Kong y.m. (2021) [32], with minor modifications. Briefly, on day 0, rats were injected subcutaneously with 0.1 ml of an emulsion (1:1, v/v) containing bovine thyroglobulin (50 μg per 100 g body weight; Sigma-aldrich, Germany) and Complete Freund's Adjuvant (CFA) (MP Biomedicals, uSa).

To enhance autoimmune sensitization, booster injections consisting of thyroglobulin emulsified in Incomplete Freund's Adjuvant (IFA) (0.1 ml) were administered subcutaneously on days 1 and 7. This immunization schedule was designed to promote sustained autoimmune activation against thyroid antigens.

on day 21, blood samples were collected from the submandibular vein to determine serum anti-thyroid peroxidase (anti-TPO) antibody levels, thereby confirming the successful induction of AIT. Following disease confirmation, animals received oral treatment with flavonoids—including DHQ, Chry, Lt, RSV, KMF, and Tm administered at a dose of 20 mg/kg body weight, either alone or in combination with Se (50 μg /kg body weight), for a 14-day treatment period.

on day 35, after completion of the treatment regimen, animals were euthanized by decapitation, and final blood samples were collected. Serum anti-TPO concentrations were subsequently analyzed to evaluate the therapeutic efficacy, as well as the progression and severity of AIT.

Experimental design and treatment groups

animals were randomly divided into the following experimental groups (n=6–8 per group):

Control – intact rats receiving vehicle only

c ontrol + Se

AIT – rats with induced AIT

AIT + Se

AIT + DHQ

AIT + DHQ + Se

AIT + Chry

AIT + Chry + Se

AIT + Lt

AIT + Lt + Se

AIT + RSV

AIT + RSV + Se

AIT + KMF

AIT + KMF + Se

AIT + Tm

AIT + Tm + Se

Bioactive compounds were employed by oral administration with help of gavage once daily for 14 consecutive days that set up after confirmation of AIT induction. Se was administered concomitantly in combined treatment groups. Dosages were selected based on previously published experimental studies and preliminary toxicity assessments.

Blood sample collection

at the end of the experimental period, rats were fasted overnight and anesthetized using light ether anesthesia. Blood samples were collected via cardiac puncture into sterile tubes and allowed to clot at room temperature. Serum was separated by centrifugation at 3,000 rpm for 15 min at 4°C and stored at -80°C until analysis.

Determination of serum hormone levels by immunoassay (ELISA)

Serum concentrations of pancreatic and metabolic hormones, including insulin, glucagon, amylin, and somatostatin, were determined using commercially available ELISA kits with immunofluorescent detection obtained from Assay Genie (Dublin, Ireland) and LSBio (vector Laboratories, newark, ca, uSa), in accordance with the manufacturers' instructions. All assays were validated for rat serum and performed following standardized analytical procedures.

hormone measurements were performed in duplicate using a calibrated microplate reader equipped with fluorescence detection. Standard curves were generated for each hormone using serial dilutions of recombinant standards provided with the respective kits. Depending on the analytical range of the assay, hormone concentrations were expressed in pg/ml or ng/ml, as specified by the manufacturers.

The analytical sensitivity, as well as intra-assay and inter-assay coefficients of variation, were within the ranges reported by the manufacturers. To minimize inter-assay variability, samples from all experimental groups were analyzed within the same assay run whenever possible. Quality control samples with known concentrations were included in each assay plate to ensure accuracy and reproducibility of the measurements.

HbA1c

hb a1c concentrations were measured using an auto - mated high-performance analyzer (HA-8180T; Arkray, Kyoto, Japan) following the manufacturer's instructions and expressed as a percentage of total hemoglobin.

hba1c values were calculated as the proportion of glycated hemoglobin relative to total hemoglobin and multiplied by 100.

Hepatic glycogen content was determined using the anthrone colorimetric method

Liver tissue samples were homogenized, and glycogen was extracted according to standard procedures. The extracts were reacted with anthrone reagent under acidic conditions, resulting in the formation of a blue-green colored complex proportional to glycogen concentration.

absorbance was measured at 620 nm using a uv-visible spectrophotometer (uv -5100, metash, Shanghai, china).

Glycogen content was calculated from a standard calibration curve prepared with known glycogen concentrations and expressed as mg of glycogen per g of wet liver tissue (mg/g).

Statistical analysis

All results are presented as mean $\pm$ SEM. Statistical analysis and data visualization were conducted using GraphPad Prism and Origin 2021. Group differences were assessed by one-way ANOVA with Tukey's multiple comparisons test. Statistical significance was defined as $P < 0.05$.

Results

In animals with experimentally induced AIT, disease progression was accompanied by pronounced morpho-functional alterations. Specifically, a marked intensification of inflammatory responses within the thyroid gland was observed, leading to significant glandular enlargement and the formation of prominent edematous structures (Fig. 2). These pathological changes were clearly visible at the macroscopic level, allowing unambiguous differentiation from healthy control animals.

in the control rats, the thyroid glands exhibited a normal macroscopic appearance, with no signs of swelling, edema, or inflammatory enlargement, indicating preserved structural integrity. In contrast, in AIT rats, the thyroid glands showed obvious enlargement, irregular contours, and edematous thickening, which were clearly and consistently evident.

This red arrows in the pictures show the anatomical location of the thyroid glands, which highlights the profound swelling and inflammatory enlargement that was observed in AIT animals. These macroscopic evidences can provide direct visible confirmation of the successful induction and progression of AIT and emphasize the marked differences between control and diseased animals.

To further identify the independent and combined impacts of selenium, additional reference groups were incorporated into the experimental design: Control + Se, and AIT + Se. The Control + Se group allowed assessment of the effects of selenium alone under physiological conditions, whereas the AIT + Se group helped enabling evaluation of selenium's isolated impacts on the autoimmune thyroiditis under the absence of flavonoid treatment. The incorporation of these groups could provide a clearer interpretation of the relative contribution of selenium and flavonoid-selenium combinations related to the observed immunomodulatory effects. In the control group, levels of anti-TPO remained low and stable on both 21 and 35 days (8.23 ± 0.11 and 9.14 ± 0.81 , respectively), which confirmed the absence of autoimmune reactivity against thyroid tissue in healthy animals.

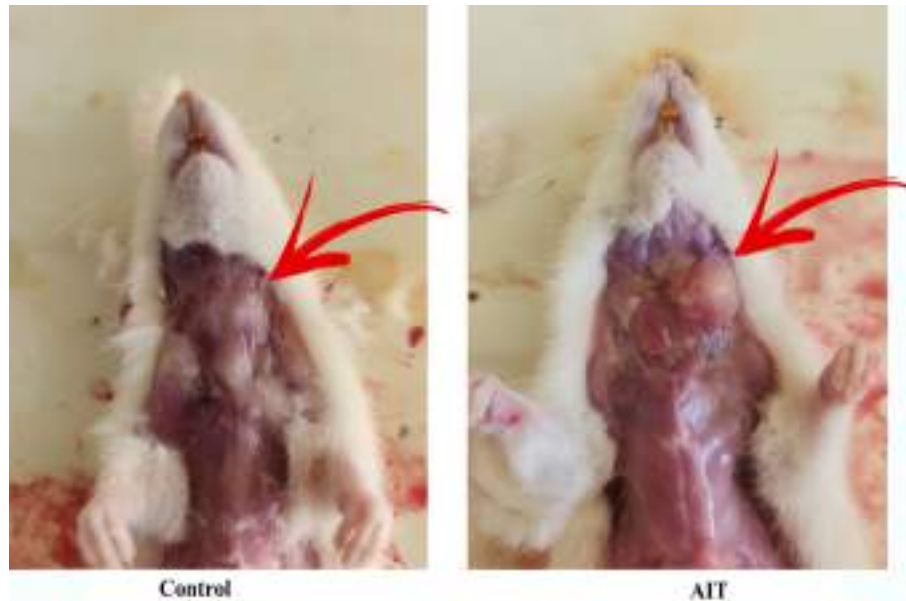


Fig. 2. Representative macroscopic appearance of the thyroid glands in control rats and rats with AIT.

A comparable finding was noted in the Control + Se group, where levels of anti-TPO remained within the physiological range and did not differ significantly from untreated controls ($P > 0.05$), which indicated that selenium alone did not lead to autoimmune activation under normal conditions. These patterns indicate that administration of selenium did not provoke autoimmune activation or alter basal anti-TPO levels in healthy animals.

In contrast, the AIT group performed an acute increase in anti-TPO activity, reaching 382.47 ± 21.31 on 21 days and further increasing to 404.46 ± 25.12 on 35 days (Fig. 3), which reflects a progressive autoimmune response relative to the thyroid peroxidase.

In the AIT + Se group, anti-TPO levels remained comparable to those observed in untreated AIT animals, indicating that selenium alone had no significant effect on anti-TPO levels. However, this decrease was less pronounced than those in groups receiving combined flavonoid and selenium therapy. These evidences related to experiments including selenium alone provides only partial protection against autoimmune activation, whereas its immunomodulatory property becomes more pronounced when combined with flavonoids presenting antioxidant and anti-inflammatory properties.

Experimental treatment with flavonoids and polyphenolic compounds resulted in a considerable reduction of levels of anti-TPO; however, the magnitude of this effect varied depending on the administered compound. In the DHQ and Chry groups, anti-TPO values at day 21 were comparable to those observed in the untreated AIT group, whereas a marked decrease was detected by day 35 (79.8

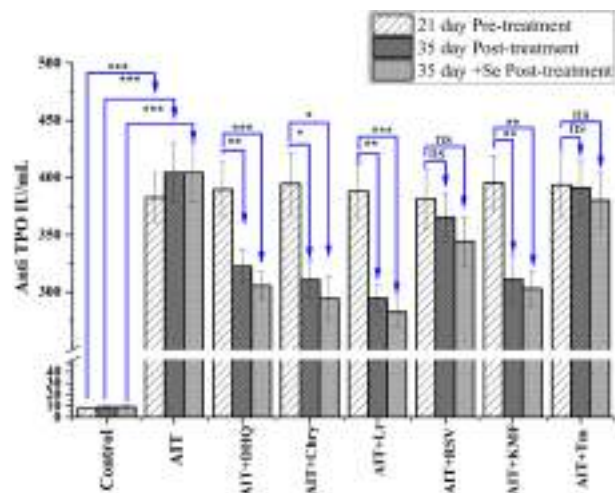


Fig. 3. Serum anti-TPO levels in control and AIT rats measured at day 21 (baseline) and day 35 (after treatment). Data are expressed as mean $\pm$ SEM ($n=6$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the AIT group.

$\pm 4.2\%$ and $76.8 \pm 4.1\%$ relative to AIT, respectively; $P < 0.05$). These effects may be attributed to the antioxidant and inflammation-modulating properties of these flavonoids.

Even more pronounced reductions in anti-TPO activity were observed in animals treated with Lt and kaempferol. On day 35, anti-TPO levels decreased to $72.9 \pm 2.8\%$ ($P < 0.01$) and $76.9 \pm 5.4\%$ ($P < 0.05$) of AIT values, respectively, suggesting a stronger immunomodulatory potential of these compounds. In the rSv-treated group,

anti-TPO activity decreased to $90.3 \pm 5.2\%$ by day 35; although a reduction was evident, the effect was less pronounced compared with other flavonoids. This may reflect resveratrol's predominant influence on antioxidant and metabolic pathways rather than a direct suppression of autoantibody synthesis.

In contrast, treatment with Tm did not result in a substantial decrease in anti-TPO levels, which remained high ($96.7 \pm 6.0\%$), indicating limited efficacy of this compound in suppressing thyroid peroxidase-directed autoimmune responses under AIT conditions. When anti-TPO activity in the AIT group on day 35 was defined as 100%, flavonoid treatment reduced this parameter to varying extents. The strongest effects were observed with Lt (-27.1%), followed by DHQ (-23.2%) and KMF (-23.1%). DHQ exerted a moderate effect (-20.2%), whereas resveratrol demonstrated relatively weak immunomodulatory activity (-9.7%). The Tm group showed values close to those of untreated AIT animals (-3.3%), which confirms its limited effectiveness in inhibiting production of anti-TPO.

Following AIT induction, flavonoids were administered intragastrically in combination with Se for 14 days. Anti-TPO activity was subsequently assessed in blood plasma using eLiSa on days 21 and 35. In the control group, anti-TPO levels were markedly lower than in the AIT group, showing reductions of approximately $97.8 \pm 0.6\%$ on day 21 and $97.7 \pm 0.5\%$ on day 35 ($P < 0.001$), thereby clearly confirming the pathological nature of changes observed in the AIT animals (Fig. 3).

Compared with the AIT group, no statistically significant differences were detected among treated groups on day 21, with values differing by only $\pm 2\text{--}4\%$ ($P > 0.05$), indicating that the effects of flavonoids and Se were not yet fully manifested during the early phase of the autoimmune process. However, by day 35, clear and statistically significant differences emerged. The combination of DHQ and Se (AIT + DHQ + Se) reduced anti-TPO levels by $24.5 \pm 3.1\%$ relative to AIT ($P < 0.05$). Compared with the untreated AIT group, anti-TPO levels decreased by 21.3% in the AIT + Chry group and by 25.4% in the AIT + Chry + Se group.

The most pronounced therapeutic effect was observed in the Lt and Se combination group (AIT + Lt + Se), where anti-TPO activity decreased by $30.1 \pm 3.2\%$ compared with AIT, reaching a high level of statistical significance ($P < 0.01$). This result highlights the superior efficacy of Lt in suppressing autoimmune processes. Resveratrol monotherapy (AIT + RSV) reduced anti-TPO levels by $14.9 \pm 4.6\%$ ($P < 0.05$), although its effectiveness was lower than that of flavonoid–Se combinations. KMF treatment (AIT + KMF) produced a more

pronounced effect, resulting in a $25.0 \pm 3.9\%$ reduction relative to AIT ($P < 0.05$).

Conversely, in the Tm-treated group (AIT + Tm), only a modest reduction of $6.0 \pm 4.8\%$ was observed, which did not reach statistical significance ($P > 0.05$), indicating limited efficacy of this compound at the administered dose and duration. The persistence of elevated anti-TPO levels under AIT conditions reflects ongoing autoimmune inflammation and oxidative stress. In contrast, combined treatment with flavonoids and Se—particularly in Lt, DHQ, and Chry-based regimens—demonstrated a clear therapeutic advantage over time.

percentage-based analysis provided a more robust assessment of treatment efficacy by minimizing dependence on absolute values and highlighting the relative protective effects of each compound under AIT conditions. Notably, the high effectiveness of the Lt+Se combination may be associated with its ability to suppress inflammatory signaling pathways and modulate immune responses.

Given that the marked elevation of anti-TPO levels under AIT conditions confirms activation of autoimmune mechanisms, the subsequent phase of the study focused on evaluating the metabolic consequences of these immune disturbances, particularly through analysis of pancreatic endocrine function and insulin secretion parameters.

Levels of plasma insulin and their modulation under the AIT

In the control animals, levels of plasma insulin displayed a modest growth (approximately 11.7%) from day 21 to day 35; however, this change did not show statistical significance ($P > 0.05$), which indicated stable activity of pancreatic β -cell secretion under physiological conditions. Administration of selenium alone (control + Se) did not significantly change levels of plasma insulin when compared with untreated controls ($P > 0.05$), thereby suggesting that selenium did not influence basal insulin secretion in the groups including healthy animals. Under the AIT conditions, levels of plasma insulin decreased by approximately 15.3% on day 35 compared with day 21 ($P < 0.05$), while plasma insulin levels significantly decreased by $15.3 \pm 4.7\%$ on day 35 compared with day 21 ($P < 0.05$), reflecting a progressive disruption of pancreatic β -cell functional activity associated with the autoimmune phenomenon. In the AIT + Se group, insulin levels showed a slight increase when compared with untreated AIT animals; however, this change did not present statistical significance ($P > 0.05$), by which selenium alone provides only limited improvement of pancreatic β -cell function.

in animals treated with DhQ, insulin levels increased markedly by $41.7 \pm 8.9\%$ on day 35 relative to day 21 ($P < 0.01$). When combined with Se, this increase reached $49.3 \pm 10.7\%$ ($P < 0.01$), indicating a time-dependent and synergistic therapeutic effect.

Treatment with Chry resulted in a moderate increase in insulin levels of $29.7 \pm 9.6\%$ ($P < 0.05$). The addition of Se slightly enhanced this effect to $31.9 \pm 11.2\%$; however, this difference did not confer a statistically significant additional benefit ($P > 0.05$), suggesting a moderate efficacy of Chry in this model (Table 1).

Lt treatment produced the most pronounced positive dynamics in insulin secretion. on day 35, plasma insulin levels increased by $60.1 \pm 11.4\%$ when compared with day 21 ($P < 0.01$). in combination with Se, this increase depicted $65.7 \pm 13.1\%$ and remained highly significant ($P < 0.001$). These results indicate that Lt is among the most effective flavonoids in restoring pancreatic β -cell secretory function under AIT conditions.

RSV treatment led to a $41.9 \pm 10.2\%$ increase in insulin levels ($P < 0.01$). although Se co-administration further increased this parameter to $48.1 \pm 11.6\%$, the overall effect remained less pronounced than that observed in the Lt-treated groups.

in KmF-treated animals, insulin levels increased by $49.6 \pm 12.3\%$ on day 35 compared with day 21 ($P < 0.01$). When combined with Se, this increase reached $62.4 \pm 14.8\%$ ($P < 0.001$), resulting in insulin concentrations approaching those of the control group. These findings suggest that KmF, particularly in combination with Se, exerts a strong regulatory effect on pancreatic β -cell function.

In contrast, treatment with Tm resulted in only a minimal increase in insulin levels ($4.6 \pm 8.9\%$), which was not statistically significant ($P > 0.05$). Se co-administration did not substantially improve insulin secretion in this group, indicating limited efficacy of Tm in correcting β -cell dysfunction under AIT conditions.

Overall, AIT was associated with a progressive decline in insulin secretion over time, reflecting a deepening

pancreatic β -cell dysfunction. Treatment with DHQ, Lt, and KmF promoted a significant time-dependent restoration of insulin secretion. co-administration with Se further potentiated these beneficial effects, particularly in the Lt and KmF groups, thereby supporting the restoration of metabolic and endocrine homeostasis under autoimmune conditions.

Alterations in circulating glucagon levels under AIT and their correction by flavonoids

At day 21 after AIT induction, circulating glucagon levels were significantly elevated by approximately 35–40% compared with the control group ($P < 0.001$), indicating pronounced dysfunction of pancreatic α -cell activity. Following treatment, measurements performed at day 35 demonstrated different degrees of correction of AIT-induced hyperglucagonemia depending on the administered flavonoid (Fig. 4). This hyperglucagonemia exhibiting dysregulation of metabolic homeostasis and impaired insulin–glucagon counter-regulatory balance under the autoimmune conditions. in the c ontrol + Se group, glucagon levels remained comparable relative to those in untreated control animals ($P > 0.05$), indicating that selenium administration alone does not influence basal α -cell activity under physiological conditions. In contrast, the AIT + Se group exhibited only a moderate fall in glucagon levels when compared with untreated AIT animals, which suggested a partial attenuation of α -cell hyperactivity; however, this impacts were less pronounced than that observed following flavonoid-based interventions.

Treatment with Lt exerted the most pronounced corrective effect on AIT-induced hyperglucagonemia, resulting in an about an 18% decrease in glucagon levels at day 35 relative to the AIT group ($P < 0.001$), with values approaching those of the control group. The relatively low standard error of the mean ($\pm \text{SEM} \approx 4\text{--}6\%$) in this group indicates a stable normalization of glucagon secretion. moreover, the Lt–Se combination further potentiated this effect, leading to a reduction of glucagon levels

Table 1. Alterations in insulin secretion in AIT and modulation by flavonoids with or without Se

animals group	21 day (ng/ml)	35 day (ng/ml)	+Se 35 day (ng/ml)
control	7.24 ± 0.29	$8.09 \pm 0.43^{**}$	8.51 ± 0.62 ns
AIT	1.57 ± 0.13	$1.33 \pm 0.02^{*}$	1.68 ± 0.1 ns
AIT+DHQ	1.44 ± 0.12	$2.04 \pm 0.04^{**}$	$2.15 \pm 0.18^{*}$
AIT+Chry	1.38 ± 0.11	$1.79 \pm 0.06^{*}$	$1.82 \pm 0.16^{*}$
AIT+Lt	1.43 ± 0.13	$2.29 \pm 0.04^{**}$	$2.37 \pm 0.24^{**}$
AIT+RSV	1.31 ± 0.09	$1.86 \pm 0.03^{*}$	$1.94 \pm 0.14^{*}$
AIT+KmF	1.41 ± 0.08	$2.11 \pm 0.05^{**}$	$2.29 \pm 0.23^{**}$
AIT+Tm	1.51 ± 0.11	1.58 ± 0.04 ns	1.62 ± 0.14 ns

Values are presented as mean $\pm$ SEM (n=6). * $P < 0.05$, ** $P < 0.01$.

of up to 21% at day 35 compared with the AIT group ($P<0.001$), and this evidence suggested effective suppression of α -cell hyperactivity (Fig. 4).

Treatment with KMF also resulted in a significant correction of hyperglucagonemia, with glucagon concentrations decreasing by approximately 20% at day 35 relative to the AIT group ($P<0.01$). The KMF + Se combination promoted this effect, achieving a decrease of up to 21%. The low SEM values (± 5 –7%) in these groups indicate stabilization of glucagon secretion within a near-physiological range, although slightly less potent than Lt, KMF demonstrated high efficacy among the tested flavonoids.

administration of DhQ produced a moderate reduction in elevated glucagon levels under AIT conditions (≈ 12 –15% at day 35, $P<0.05$). however, the relatively higher variability ($\pm \text{SEM} \approx 8$ –10%) suggests lower consistency of the effect compared with Lt and KMF. Combination with Se enhanced the glucagon-lowering effect to approximately 19% ($P<0.01$), although normalization toward control values remained incomplete.

Treatment with Chry resulted in a 15–18% decrease in glucagon levels at day 35 compared with the AIT group ($P<0.05$). Despite a slightly greater reduction ($\sim 20\%$) observed in the c hry + Se group, the relatively wide SEM (± 9 –11%) indicates considerable inter-individual variability, suggesting limited stability of α -cell regulation in this group.

RSV exhibited one of the weakest effects on glucagon secretion, with a reduction limited to approximately 10% at day 35 ($P<0.05$). although combination with Se improved the reduction to about 16%, glucagon levels remained elevated relative to the AIT group, indicating insufficient normalization.

Tm, both alone and in combination with Se, failed to achieve effective normalization of glucagon levels. The noted reductions did not exceeding 3% at day 35, and the relatively high SEM values (± 10 –12%) displayed persistent α -cell hyperactivity and limited therapeutic efficacy in correcting AIT-associated hyperglucagonemia.

Overall, these findings demonstrate that AIT-induced hyperglucagonemia can be corrected to varying degrees by flavonoid treatment. Lt and KMF, particularly in combination with Se, exhibited the greatest efficacy by not only reducing glucagon levels relative to the AIT group but also restoring them toward the physiological range. In contrast, the remaining flavonoids produced partial and less stable effects, failing to fully suppress α -cell hyperactivity under AIT conditions.

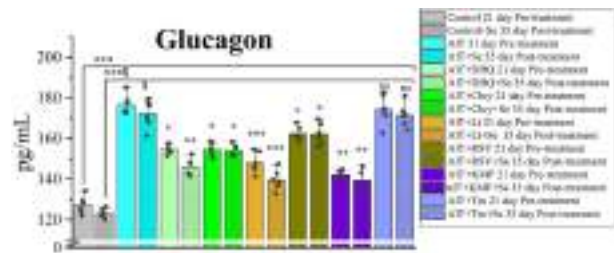


Fig. 4. Alterations in glucagon secretion in AIT and modulation by flavonoids with or without Se measured at day 21 (baseline) and day 35 (after treatment). values are presented as mean $\pm$ SEM ($n=6$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared with the AIT group.

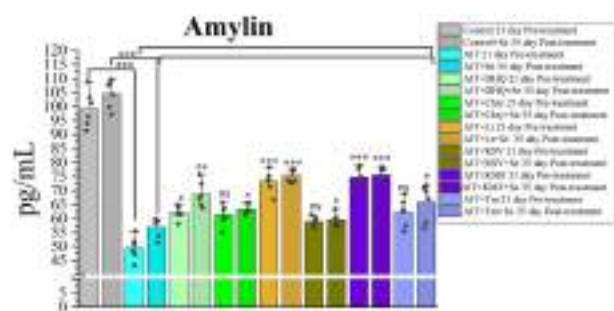


Fig. 5. Alterations in amylin secretion in AIT and modulation by flavonoids with or without Se measured at day 21 (baseline) and day 35 (after treatment). values are presented as mean $\pm$ SEM ($n=6$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared with the AIT group.

Alterations in serum amylin levels under AIT

in control rats, serum amylin concentrations determined by iFa remained stable throughout the 21–35 day observation period, averaging 99.1 ± 2.6 pg/ml at both time points (day 21 and day 35). Minor fluctuations observed during this period were not statistically significant ($P>0.05$), indicating preserved secretory activity of pancreatic β -cells under physiological conditions (Fig. 5).

administration of selenium (control + Se) did not significantly vary levels of serum amylin compared with untreated controls ($P>0.05$), which suggested that selenium did not influence basal amylin secretion in healthy animals. In contrast, under AIT conditions, serum amylin levels at day 35 demonstrated a significant decline of $-50.1 \pm 4.2\%$ relative to the control group ($P<0.001$), and this reflected substantial dysfunction of pancreatic β -cell function associated with autoimmune-mediated metabolic dysregulation. In the AIT + Se group, concentration of serum amylin showed only a moderate increase when compared with untreated AIT animals, by which a partial recovery of β -cell secretory activity was described; however, this improvement remained less pronounced than those treated following flavonoid treatment.

Amylin levels at day 35 increased by $+24.9 \pm 5.1\%$ relative to the AIT group ($P < 0.05$) in animals treated with DhQ. A combination of DhQ and Se further potentiated this recovery, it led to a $+37.0 \pm 6.3\%$ increase in serum amylin concentration ($P < 0.01$), suggesting enhanced restoration of β -cell secretory capacity.

In the chry-treated group, amylin levels at day 35 increased by $+24.7 \pm 4.8\%$ compared with the AIT group, while combination with Se led to a slightly greater improvement of $+28.1 \pm 4.2\%$ ($P < 0.05$). These findings indicate a moderate but consistent protective effect of Chry on β -cell function.

Treatment with Lt resulted in a profound elevation of serum amylin levels at day 35 by $+48.0 \pm 5.9\%$ relative to AIT ($P < 0.001$). The Lt-Se combination demonstrated the most robust effect, achieving near-maximal recovery with an increase of $+51.5 \pm 4.1\%$ ($P < 0.001$), indicating highly effective restoration of pancreatic β -cell secretory activity.

In the resveratrol-treated group, amylin levels at day 35 increased by $+18.8 \pm 3.6\%$, while co-administration with Se slightly enhanced this effect to $+20.2 \pm 3.1\%$ ($P < 0.05$). Despite statistical significance, the degree of recovery remained limited compared with Lt- and kaempferol-based interventions.

Administration of kaempferol significantly increased serum amylin levels at day 35 by $+50.3 \pm 5.4\%$, and the kaempferol-Se combination further improved this response to $+51.6 \pm 4.0\%$ ($P < 0.001$). The magnitude and consistency of this effect indicate strong β -cell functional recovery approaching physiological levels.

In the Tm-treated group, amylin concentrations at day 35 increased by $+27.1 \pm 5.2\%$ relative to AIT, while co-treatment with Se resulted in a more significant refinement of $+33.0 \pm 6.1\%$ ($P < 0.05$). But, the observed variability suggests incomplete normalization of β -cell activity.

Overall, these findings demonstrate that AIT induces a profound suppression of amylin secretion, reflecting severe pancreatic β -cell dysfunction. Flavonoid treatment resulted in varying degrees of functional recovery, with Lt and kaempferol, particularly in combination with Se, showing the greatest efficacy in restoring amylin secretion toward physiological levels. Other flavonoids exerted partial or moderate effects, indicating limited capacity to fully reverse AIT-associated β -cell impairment.

Alterations in serum somatostatin levels under AIT (IFA)

To assess the progression of δ -cell dysfunction and the modulatory effects of flavonoids and Se under AIT,

levels of plasma somatostatin on day 35 were analyzed relative to the 21-day AIT baseline.

In the AIT + Se group, concentration of plasma somatostatin showed a similar increase relative to the day-21 AIT baseline ($+2.5 \pm 2.1\%$, $P > 0.05$), and it indicated that selenium alone was insufficient to restore δ -cell secretory activity under autoimmune conditions.

In rats with AIT at day 21, plasma somatostatin was considered the baseline reference (100%), reflecting established autoimmune-mediated suppression activity of secretion of δ -cell (Fig. 6).

In untreated AIT rats at day 35, somatostatin levels showed only a negligible change compared with day 21 ($+2.5 \pm 2.1\%$, $P > 0.05$), exhibiting the absence of spontaneous recovery of δ -cell function during disease progression.

Administration of DHQ (AIT + DHQ, day 35) resulted in a marked elevation of plasma somatostatin by $24.1 \pm 4.6\%$ relative to the day-21 AIT baseline ($P < 0.01$), demonstrating a significant restoration of inhibitory endocrine signaling.

Notably, combined treatment with DHQ and Se (AIT + DHQ + Se, day 35) further potentiated this effect, leading to a $31.6 \pm 5.1\%$ increase in somatostatin levels compared with day 21 ($P < 0.001$). This response was significantly greater than that observed with DHQ alone ($P < 0.05$), indicating a synergistic or additive interaction between DhQ and Se.

In animals treated with Chry (AIT + Chry, day 35), plasma somatostatin increased by $9.0 \pm 3.8\%$ relative to day 21 ($P < 0.05$). Co-administration of chry with Se (AIT + Chry + Se, day 35) enhanced this response to

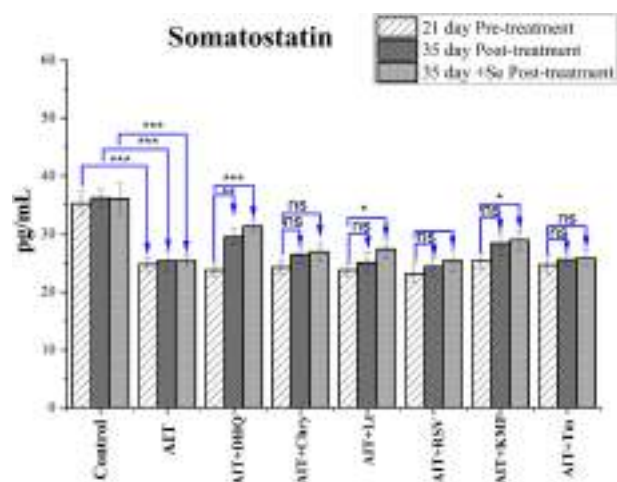


Fig. 6. Alterations in somatostatin secretion in AIT and modulation by flavonoids with or without Se measured at day 21 (baseline) and day 35 (after treatment). Values are presented as mean $\pm$ SEM ($n=6$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the AIT group.

$13.7 \pm 4.2\%$ ($P < 0.05$ vs day 21), although the difference compared with chry monotherapy did not reach statistical significance ($P > 0.05$).

Treatment with KMF (AIT + KMF, day 35) produced a moderate but significant increase in somatostatin levels ($+11.8 \pm 4.9\%$, $P < 0.05$ vs day 21). This effect was further amplified by Se co-treatment (AIT + KMF + Se, day 35), resulting in a $17.9 \pm 4.8\%$ elevation relative to baseline ($P < 0.01$), suggesting a Se-mediated enhancement of KMF efficacy.

In contrast, Lt treatment (AIT + Lt, day 35) induced only a modest, non-significant change in somatostatin levels ($+5.3 \pm 4.1\%$, $P > 0.05$). Addition of Se (AIT + Lt + Se, day 35) did not substantially improve this response ($+7.4 \pm 3.9\%$, $P > 0.05$), indicating limited δ -cell responsiveness to Lt regardless of Se supplementation.

Similarly, RSV (AIT + RSV, day 35) failed to restore somatostatin secretion ($-3.4 \pm 3.6\%$, $P > 0.05$), and Se co-treatment (AIT + RSV + Se, day 35) did not alter this outcome ($-1.2 \pm 3.5\%$, $P > 0.05$).

Finally, Tm monotherapy (AIT + Tm, day 35) resulted in a minimal and non-significant increase in somatostatin ($+3.6 \pm 3.2\%$, $P > 0.05$). Combined administration with Se (AIT + Tm + Se, day 35) produced a similarly modest response ($+6.1 \pm 3.4\%$, $P > 0.05$), suggesting negligible interaction between Tm and Se in regulating δ -cell activity.

Relative to the day-21 AIT baseline, somatostatin secretion does not recover spontaneously by day 35. DHQ exhibits the strongest restorative effect, which is further potentiated by Se, indicating a synergistic improvement of δ -cell regulatory function. KMF and Chry show moderate efficacy, with partial enhancement upon Se co-administration, whereas Lt, RSV, and Tm—either alone or combined with Se—fail to induce statistically significant recovery. These findings underscore the compound-specific nature of flavonoid–Se interactions in modulating somatostatin signaling under autoimmune conditions.

HbA1c levels under AIT and flavonoid–Se interventions

To evaluate long-term glycemic control under AIT and therapeutic interventions, HbA1c levels were identified on day 21 and day 35 and expressed as a percentage (%) of total hemoglobin (Fig. 7).

In control rats, hba1c levels remained stable throughout the experimental period, showing no significant changes between day 21 and day 35, either in untreated conditions or in following Se administration ($P > 0.05$). In the control + Se group, concentration of hba1c remained comparable to those in untreated control animals

($P > 0.05$), thereby indicating that selenium administration alone did not significantly affect control of long-term glycemic in healthy rats. This finding confirms preserved glycemic homeostasis in healthy animals.

In contrast, rats with AIT exhibited a pronounced elevation of hba1c already at day 21, indicating sustained hyperglycemic stress. By day 35, hba1c levels in untreated AIT animals showed a slight additional increase of $+2.4 \pm 1.9\%$ relative to day 21, which did not reach statistical significance ($P > 0.05$), suggesting persistence of impaired glycemic regulation without spontaneous improvement. In the AIT + Se group, HbA1c levels were kept comparable to those in untreated AIT animals ($P > 0.05$), by which selenium alone did not substantially improve long-term glycemic control under autoimmune conditions.

Treatment with dihydroquercetin (AIT + DHQ) resulted in a significant reduction of HbA1c by $-8.6 \pm 2.3\%$ at day 35 compared with the day-21 AIT baseline ($P < 0.01$), indicating marked improvement in long-term glycemic control. Co-administration of Se (AIT + DHQ + Se) further enhanced this effect, leading to a $-9.5 \pm 2.7\%$ decrease relative to day 21 ($P < 0.01$), although the difference between DHQ alone and DHQ + Se was not statistically significant ($P > 0.05$).

In the chrysoeriol-treated group (AIT + Chry), HbA1c levels decreased by $-4.5 \pm 3.1\%$ at day 35 ($P < 0.05$ vs day 21). The addition of Se (AIT + Chry + Se) produced a comparable reduction ($-4.9 \pm 2.8\%$, $P < 0.05$), indicating a modest but consistent improvement in glycemic status.

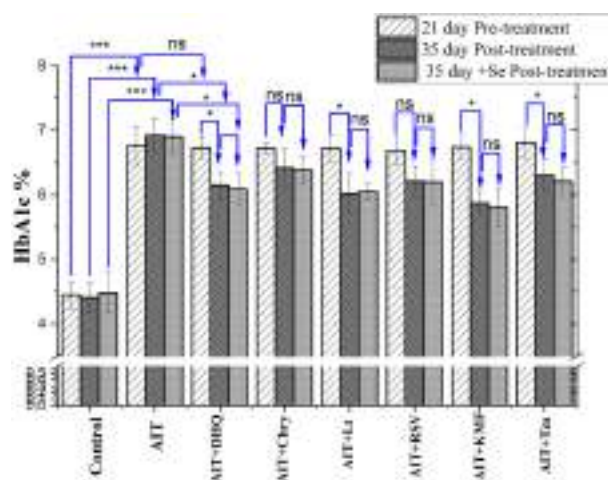


Fig. 7. Changes in HbA1c levels in AIT and modulation by flavonoids with or without Se measured at day 21 (baseline) and day 35 (after treatment). Values are presented as mean $\pm$ SEM ($n=6$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the AIT group.

Administration of Lt (AIT + Lt) induced a more pronounced decrease in HbA1c ($-10.4 \pm 3.4\%$) by day 35 ($P < 0.01$ vs day 21). Se co-treatment (AIT + Lt + Se) maintained this improvement ($-10.0 \pm 2.9\%$, $P < 0.01$), suggesting that Lt exerts a strong glucose-lowering effect that is not substantially augmented by Se.

In contrast, RSV treatment (AIT + RSV) led to only a minor and statistically non-significant reduction of HbA1c ($-7.2 \pm 4.1\%$; $P > 0.05$). Se supplementation (AIT + RSV + Se) did not provide additional benefit ($-7.3 \pm 4.6\%$; $P > 0.05$), indicating limited efficacy of RSV in improving long-term glycemic control under AIT conditions.

Treatment with kaempferol (AIT + KMF) significantly reduced HbA1c levels by $-12.9 \pm 2.5\%$ at day 35 compared with day 21 ($P < 0.001$). This effect was slightly enhanced by Se co-administration (AIT + KMF + Se), resulting in a $-13.8 \pm 3.1\%$ decrease ($P < 0.001$), identifying kaempferol as one of the most effective interventions for hb a1c normalization.

Finally, Tm treatment (AIT + Tm) produced a moderate but significant reduction in HbA1c ($-7.5 \pm 3.0\%$; $P < 0.05$). The addition of Se (AIT + Tm + Se) did not significantly strengthen this effect ($-8.8 \pm 3.2\%$; $P < 0.05$).

Relative to the day-21 AIT baseline, HbA1c levels remained persistently elevated in untreated animals, indicating sustained dysregulation of long-term glycemic control. among the tested interventions, kaempferol and Lt demonstrated the strongest HbA1c-lowering effects, followed by dihydroquercetin, whereas chrysoeriol and Tm exerted moderate efficacy. RSV showed limited impact, and Se supplementation provided only modest or supportive benefits, mainly enhancing already effective flavonoid treatments rather than acting independently.

Hepatic glycogen content in rat liver homogenates (mg/g wet tissue)

in control rats, hepatic glycogen content remained stable between day 21 and day 35 (47.25 ± 1.79 vs. 48.19 ± 2.65 mg/g wet tissue), showing a minimal and statistically insignificant increase of $+2.0 \pm 3.1\%$ ($P > 0.05$). Se supplementation did not significantly alter hepatic glycogen stores in control animals (49.08 ± 2.88 mg/g, $+1.8 \pm 2.9\%$ vs. day 35, $P > 0.05$), and this result explained preserved glycogen homeostasis under physiological conditions (Fig. 8).

In contrast, AIT was associated with a pronounced depletion of hepatic glycogen stores. on day 21, glycogen levels in AIT rats were reduced by $-34.9 \pm 4.6\%$ compared with controls ($P < 0.001$). This reduction persisted on day 35 (28.36 ± 1.96 mg/g), corresponding to a $-41.2 \pm 5.1\%$ decrease relative to control animals ($P < 0.001$). Se treatment failed to restore hepatic glyco-

gen in the AIT group (30.46 ± 1.60 mg/g), with levels remaining $-38.0 \pm 4.9\%$ lower than controls ($P < 0.001$).

Effect of flavonoid treatment on hepatic glycogen dynamics

DhQ treatment resulted in a marked recovery of hepatic glycogen from day 21 to day 35, with an increase of $+25.4 \pm 6.2\%$ ($P < 0.01$). Se co-administration further enhanced glycogen accumulation to 37.31 ± 1.59 mg/g, corresponding to an additional $+2.0 \pm 3.5\%$ relative to day 35 ($P > 0.05$). overall, glycogen levels increased by $+27.9 \pm 6.8\%$ compared with untreated AIT rats ($P < 0.01$).

Between day 21 and day 35, chrysoeriol treatment led to a moderate increase in hepatic glycogen of $+7.0 \pm 4.1\%$ ($P < 0.05$). Se supplementation produced a further $+1.5 \pm 3.6\%$ increase ($P > 0.05$). Despite improvement, glycogen levels remained significantly lower than control values ($P < 0.01$).

Lt administration significantly restored hepatic glycogen content, increasing levels by $+16.3 \pm 5.4\%$ from day 21 to day 35 ($P < 0.01$). Se co-treatment resulted in an additional $+3.8 \pm 3.9\%$ increase ($P < 0.05$). compared with untreated AIT rats, total recovery reached $+28.2 \pm 6.1\%$ ($P < 0.001$).

rSv treatment induced a moderate increase in hepatic glycogen of $+17.9 \pm 4.9\%$ between day 21 and day 35 ($P < 0.05$). Se supplementation slightly enhanced this effect ($+3.3 \pm 3.6\%$, $P > 0.05$). however, glycogen levels remained markedly reduced compared with controls ($P < 0.01$).

KmF demonstrated one of the strongest restorative effects, with hepatic glycogen increasing by $+30.6 \pm$

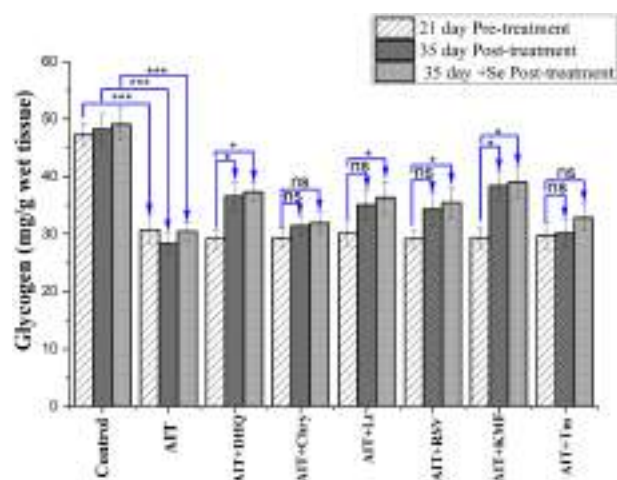


Fig. 8. Changes in hepatic glycogen content in AIT and modulation by flavonoids with or without Se measured at day 21 (baseline) and day 35 (after treatment). values are presented as mean $\pm$ SEM (n=6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the AIT group.

6.7% from day 21 to day 35 ($P < 0.001$). Se further augmented this response ($+1.6 \pm 3.4\%$, $P > 0.05$). Compared with untreated AIT rats, total recovery reached $+37.3 \pm 7.1\%$ ($P < 0.001$), approaching physiological levels.

Tm treatment resulted in only a minor increase in hepatic glycogen ($+1.6 \pm 3.8\%$, day 21 to day 35, $P > 0.05$). Se supplementation modestly improved glycogen levels ($+8.9 \pm 4.6\%$, $P < 0.05$), but overall recovery remained limited and significantly lower than control values ($P < 0.01$).

Collectively, these results demonstrate that AIT induces a substantial depletion of hepatic glycogen stores. Among the tested flavonoids, KMF and Lt, particularly in combination with Se, showed the greatest efficacy in restoring liver glycogen content toward physiological values. Other flavonoids exerted partial or inconsistent effects, indicating limited capacity to fully reverse AIT-associated hepatic glycogen depletion.

The present study demonstrates that AIT induces profound disturbances in pancreatic endocrine function and hepatic glucose metabolism, extending the pathological impact of AIT beyond the thyroid gland. The observed reduction in insulin and amylin secretion, accompanied by persistent hyperglucagonemia, elevated HbA1c levels, and depletion of hepatic glycogen stores, indicates a systemic metabolic dysregulation driven by autoimmune inflammation and oxidative stress.

Pancreatic β -cells are particularly vulnerable to inflammatory mediators and redox imbalance. In this study, the marked suppression of insulin and amylin secretion under AIT conditions reflects progressive β -cell dysfunction, which likely contributes to impaired glucose utilization and long-term glycemic instability. Concurrently, excessive glucagon secretion suggests α -cell hyperactivity, further promoting hepatic glycogenolysis and exacerbating hyperglycemic stress.

Flavonoid treatment resulted in compound-specific modulation of these endocrine disturbances. Among the tested compounds, Lt and KmF exhibited the most pronounced restorative effects on pancreatic hormone secretion and hepatic glycogen metabolism. These effects may be attributed to their well-documented ability to suppress NF- κ B and JAK/STAT signaling pathways, reduce pro-inflammatory cytokine production, and preserve mitochondrial function within endocrine cells. Restoration of insulin–glucagon balance under these treatments was closely associated with improved hepatic glycogen storage, underscoring the functional coupling between pancreatic endocrine regulation and liver glucose metabolism.

The addition of Se further potentiated the efficacy of selected flavonoids, particularly Lt and KMF. Se is a

critical component of antioxidant selenoproteins and plays an essential role in maintaining redox homeostasis and thyroid hormone metabolism. The observed synergistic effects suggest that combined flavonoid–Se therapy enhances cellular antioxidant capacity, thereby protecting endocrine tissues from autoimmune-mediated oxidative damage.

In contrast, RSV and Tm demonstrated limited capacity to restore endocrine and metabolic parameters, indicating that not all polyphenolic compounds exert equivalent protective effects under autoimmune conditions. These findings highlight the importance of compound-specific mechanisms and reinforce the need for targeted selection of bioactive agents in the management of autoimmune-associated metabolic dysfunction.

Overall, this study provides integrative evidence that pancreatic endocrine dysfunction and hepatic glycogen depletion are key metabolic consequences of AIT and that selective flavonoid–Se combinations can effectively counteract these disturbances.

Discussion

This study aims to demonstrate that AIT induces profound disturbances not only in thyroid function but also in pancreatic endocrine activity and hepatic glucose metabolism, thereby highlighting the systemic pathological potential of AIT beyond the thyroid gland. The observed reduction in insulin and amylin secretion following AIT induction was accompanied by multiple metabolic abnormalities, including hyperglucagonemia, elevated HbA1c levels, and depletion of hepatic glycogen stores. Collectively, these alterations indicate the gradual development of systemic metabolic dysregulation driven by autoimmune inflammation and oxidative stress.

Pancreatic β -cells are known to be highly vulnerable and particularly sensitive to inflammatory mediators and redox imbalance. In the present study, marked suppression of insulin and amylin secretion under AIT conditions reflects severe β -cell dysfunction, which likely contributes to impaired glucose utilization and long-term glycemic instability. Conversely, excessive glucagon secretion indicates α -cell hyperactivity, which not only promotes hepatic glycogenolysis but also exacerbates hyperglycemic stress, further aggravating metabolic imbalance [33].

Among the investigated flavonoid treatments, Lt and KmF deserve special attention, as they exhibited the most pronounced restorative effects on pancreatic hormone secretion and hepatic glycogen metabolism. These beneficial effects can be attributed to their well-docu-

mented antioxidant and anti-inflammatory properties. Based on previous experimental studies, modulation of inflammatory pathways may be involved in such effects, these pathways are NF- κ B and JAK/STAT signaling. However, these pathways were not directly assessed in the present study and should be regarded as plausible mechanistic interpretations rather than direct experimental findings. Through suppression of proinflammatory cytokine production and preservation of mitochondrial function within endocrine cells, these flavonoids may effectively counteract autoimmune-mediated cellular damage [34]. The restoration of the insulin–glucagon balance is accompanied by replenishment of hepatic glycogen stores, and underscores the close functional interplay between pancreatic endocrine regulation and liver glucose metabolism.

In turn, the addition of Se further enhanced the therapeutic efficacy of flavonoids such as Lt and KMF. Se, a key constituent of antioxidant selenoproteins, plays a crucial role in maintaining redox homeostasis and regulating thyroid hormone metabolism [35]. The observed synergistic effects claim that flavonoid–Se combinations maintain cellular antioxidant defenses, by which endocrine tissues are protected from autoimmune-driven oxidative injury [36]. In contrast, RSV and Tm demonstrated comparatively limited efficacy in restoring endocrine and metabolic parameters, as a result it highlights the significance of compound-specific mechanisms when selected bioactive agents for intervention under AIT conditions. The various efficacy of the investigated flavonoids may also be expressed by variations in their chemical structure and substitution patterns. Structure–activity relationship studies indicate that the number and position of hydroxyl groups in the flavonoid backbone have an influence on antioxidant capacity, radical scavenging ability, and also interaction with intracellular signaling pathways. Flavonoids, such as, luteolin and kaempferol, contain favorable hydroxylation patterns that help enhance their redox-modulating and anti-inflammatory properties, which may partially shed light on their superior efficacy in restoring pancreatic hormone secretion and hepatic glycogen metabolism when compared with other tested compounds [37].

Since the flavonoids in this study were used by oral administration, their intestinal absorption and systemic bioavailability must also be emphasized when interpreting their biological efficacy. Flavonoids are absorbed in the small intestine mainly by enzymatic hydrolysis and may experience extensive phase II metabolism (including glucuronidation, sulfation, and methylation within the intestinal mucosa and liver). Experimental pharmacokinetic studies have noted that luteolin, one of

the rapidly absorbing flavonoids across the intestinal epithelium, is converted predominantly into glucuronide conjugates, although a fraction of the aglycone may escape intestinal metabolism, and it can enter systemic circulation. These circulating metabolites are able to keep biological activity and promote the antioxidant and anti-inflammatory properties of luteolin [38].

The differences in their biological efficacy observed in the present study may be described by structural differences between the used flavonoids. Luteolin and kaempferol are regarded as aglycone flavonoids with relatively favorable lipophilicity and as multiple hydroxyl groups in the B ring, which mitigates passive diffusion across intestinal membranes and refines systemic bioavailability [39]. Kaempferol has been claimed to be efficiently absorbed in the small intestine, and subsequently faces hepatic phase II metabolism, generating glucuronide and sulfate conjugates. These can circulate in plasma in order to maintain biological activity. These pharmacokinetic properties may partially explain the basic concept of why luteolin and kaempferol produced the most pronounced improvements in pancreatic hormone secretion and hepatic glycogen metabolism in this study [25].

In contrast, dihydroquercetin (taxifolin) presented only moderate efficacy. Though it consists of multiple hydroxyl groups (OH) that is able to provide strong antioxidant potential, the membrane permeability may be decreased by its lower lipophilicity and structural saturation of the C2–C3, and taxifolin's unit limits intestinal absorption compared with flavones such as luteolin and kaempferol. Consequently, systemic availability of dihydroquercetin may not be higher, and this compound could partly account for its intermediate biological effect [40].

An even more limited efficacy was recorded for chrysoeriol and thamiflaside. Chrysoeriol (3'-methoxyapigenin or luteolin 3'-methyl ether) contains a methoxy substitution that reduces a large amount of free hydroxyl groups for radical scavenging, and may vary interactions with cellular signaling pathways [41]. Furthermore, thamiflaside is regarded as a complex flavonoid glycoside (apigenin 7-O- α -L-2''-methoxyrhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside), and prior hydrolysis is required by glycosylated flavonoids, and this may occur by intestinal enzymes or microbiota before absorption [42]. Intestinal permeability and systemic bioavailability can be reduced by an additional metabolic step, thereby limiting compounds' activity *in vivo*.

It should also be noted that a relatively novel flavonoid-based compound is represented by thamiflaside, and currently available pharmacological and pharmaco-

kinetic findings regarding its absorption, metabolism, and systemic biological effects are limited.

In addition to direct antioxidant and anti-inflammatory mechanisms, other regulatory pathways may also contribute to the observed endocrine improvements. Although GLP-1 levels were not directly assessed in the present study, it can be hypothesized that impairment of the incretin system may play a role in pancreatic endocrine dysfunction associated with AIT. Chronic autoimmune inflammation and oxidative stress are known to disrupt gut–pancreas crosstalk, which potentially impacts GLP-1–mediated insulinotropic signaling [43]. In these situations, the restoration of pancreatic hormone secretion observed following flavonoid treatment may be partly mediated through modulation of incretin-related pathways, a possibility that guarantees further investigation, identification.

The observed improvement and refinement in pancreatic hormone secretion and glucose homeostasis in this study are likely mediated through several complementary mechanisms. On one hand, flavonoid treatment significantly helped in preventing anti-TPO levels, thereby depending on attenuation of thyroid-specific autoimmune inflammation. Reduction of autoimmune activity in the thyroid gland may diminish systemic inflammatory and signals of oxidative stress that negatively have effects on pancreatic endocrine function. On the other hand, flavonoids might possess to exert direct cytoprotective and antioxidant effects on endocrine tissues. Thus, the observed restoration of insulin and amylin secretion may also express direct protection of pancreatic β -cells and refinement of endocrine cell function. Taken together, the metabolic improvements observed in this study are likely attributable to a combination of reduced autoimmune inflammation and direct protective influences of flavonoids on endocrine tissues. Overall, the present findings indicate that pancreatic endocrine dysfunction and hepatic glycogen depletion represent integral metabolic consequences of AIT. Moreover, therapeutic strategies based on flavonoid–Se combinations possess capability of addressing a broad spectrum of endocrine and metabolic disturbances linked with AIT.

Limitations

numerous limitations of the present study should also be admitted. Firstly, circulating thyroid hormone levels (TSH, T3, and T4) have not direct measurements. Therefore, the relative contribution of thyroid hormone imbalance and autoimmune inflammatory processes related to the observed metabolic disturbances cannot be completely separated. Secondly, inflammatory signaling

pathways including NF- κ B and JAK/STAT were not evaluated in a direct way. Finally, although the experimental model was based on thyroglobulin-induced autoimmune thyroiditis, the potential contribution of non-specific systemic inflammatory activation associated with adjuvant-induced immune stimulation has not been completely excluded. Future studies integrating thyroid hormone profiling, inflammatory pathway analysis, and pharmacokinetic evaluation of flavonoid metabolites will help clarifying these mechanisms in a clear way.

Conclusions

This study describes that autoimmune thyroiditis (AIT) trigger not only thyroid dysfunction, but also significant disturbances in pancreatic endocrine activity and metabolism of hepatic glucose. Significant changes were observed in several essential metabolic parameters, including fall of insulin and amylin secretion, increase of glucagon activity, impairment of somatostatin regulation, elevation of d hb a1c levels, and substantial depletion of hepatic glycogen stores in AIT conditions. These changes depict the development of systemic metabolic dysregulation linked with autoimmune inflammation.

Flavonoid treatments displayed compound-specific protective effects related to pancreatic endocrine function and glucose metabolism among the investigated interventions. Luteolin and kaempferol were able to demonstrate the most serious restorative effects, whereas dihydroquercetin showed moderate efficacy. Moreover, chrysoeriol and thamiflaside produced comparatively limited responses. These differences may depend on variations in chemical structure, intestinal absorption, and systemic bioavailability of the tested flavonoids.

Importantly, the combination of flavonoids with selenium has an ability of enhancing metabolic stabilization, and it offers synergistic antioxidant and immunomodulatory effects. Collectively, these findings encourage the concept that AIT should be considered not only to be a thyroid-specific disorder but to be a systemic pathological condition impacting numerous endocrine and metabolic pathways including pancreatic hormone regulation and hepatic glucose homeostasis.

Declaration of Generative AI in Scientific Writing

minimal assistance from QuillBot was used only for paraphrasing selected phrases, while all scientific information, interpretations, and conclusions were independently developed by the authors.

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