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The methanolic extracts of *Teucrium polium* L. and *Micromeria fruticosa* (L.) Druce subsp. *brachycalyx* P. H. Davis improve diabetes in streptozotocin/nicotinamide-induced type 2 diabetic female Sprague Dawley rats

Sinan Sermet [a], Muhammet Emin Cam [b, c, d, e, f, g, *], Ayse Nur Hazar-Yavuz [b], Turgut Taskin [h], Gul Sinemcan Kabatas [i], Yusufhan Yazir [i, j, k], Levent Kabasakal [b], Hatice Kubra Elcioglu [b]

[a] *Department of Clinical Sciences, Department of Pharmacology and Clinical Pharmacology, Istinye University, 34396, Istanbul, Turkey*
[b] *Department of Pharmacology, Marmara University, 34854, Istanbul, Turkey*
[c] *Center for Nanotechnology and Biomaterials Application and Research, Marmara University, 34722, Istanbul, Turkey*
[d] *Genetic and Metabolic Diseases Research and Investigation Center, Marmara University, 34854, Istanbul, Turkey*
[e] *Biomedical Engineering Department, University of Aveiro, 3810-193, Aveiro, Portugal*
[f] *Department of Pharmacology, Istanbul Kent University, 34406, Istanbul, Turkey*
[g] *MecNano Technologies, Cube Incibation, Teknopark Istanbul, 34906, Istanbul, Turkey*
[h] *Department of Pharmacognosy, Marmara University, 34854, Istanbul, Turkey*
[i] *Center for Stem Cell and Gene Therapies Research and Practice, Kocaeli University, 41001, Kocaeli, Turkey*
[j] *Department of Stem Cell, Institute of Health Sciences, Kocaeli University, 41001, Kocaeli, Turkey*
[k] *Department of Histology and Embryology, Faculty of Medicine, Kocaeli University, 41001, Kocaeli, Turkey*

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ABSTRACT

Background: *Teucrium polium* and *Micromeria fruticosa* subspecies are known to be used in traditional medicine for diabetes treatment.
Objective: The present study aimed to examine the potential antidiabetic effects and the mechanisms of antidiabetic actions of *Teucrium polium* L. methanolic extract (TP) and *Micromeria fruticosa* (L.) Druce subsp. *brachycalyx* P. H. Davis methanolic extract (MF) on rats with type 2 diabetes mellitus (DM).
Methods: Streptozotocin (STZ) and nicotinamide (NA) were injected intraperitoneally to induce type 2 diabetes mellitus (T2DM) in rats. Plant extracts' antidiabetic mechanisms of action were investigated with biochemical analyses and histopathological analyses performed.
Results: TP and MF treatments reduced the blood glucose levels compared to the untreated diabetic rats. TP reduced TNF-α levels in serum, increased insulin levels in serum and pancreas, reduced SGLT-2 levels in kidneys, reduced GLUT-2 levels in the ileum; and MF reduced TNF-α levels, and increased insulin levels in serum and pancreas, increased GLP-1 levels, and reduced GLUT-2 levels in the ileum, and reduced SGLT-2 levels in kidneys. Treatments improved the histopathological results in the pancreas, kidney, and liver.

* Corresponding author. Department of Pharmacology, Marmara University, Başıbüyük Yolu 34854 4/A Başıbüyük/Maltepe, Istanbul, Turkey.
E-mail addresses: sinan.sermet@istinye.edu.tr (S. Sermet), muhammet.cam@marmara.edu.tr (M.E. Cam), ayse.hazar@marmara.edu.tr (A.N. Hazar-Yavuz), turguttaskin@marmara.edu.tr (T. Taskin), gulsinemcan@hotmail.com (G.S. Kabatas), yusufhan.yazir@kocaeli.edu.tr (Y. Yazir), lkabasakal@marmara.edu.tr (L. Kabasakal).

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Conclusions: The results presented in this study demonstrated that TP and MF both have potential antidiabetic effects and may be effective in T2DM treatment.

Abbreviations

ANOVA	Analysis of variance
BGL	Blood glucose level
C	Control group
DM	Diabetes mellitus
DM group	Streptozotocin/nicotinamide-induced type 2 diabetic rats given saline (10 mg/5 ml/kg/day, i.g.)
DM + EM	Streptozotocin/nicotinamide-induced type 2 diabetic rats treated with empagliflozin (10 mg/kg b.w., i.g.)
DM + MF	Streptozotocin/nicotinamide-induced type 2 diabetic rats treated with *Micromeria fruticosa* (L.) Druce subsp. *brachycalyx* P. H. Davis methanolic extract (200 mg/kg b.w., i.g.)
DM + TP	Streptozotocin/nicotinamide-induced type 2 diabetic rats treated with TP *Teucrium polium* L. methanolic extract (200 mg/kg b.w., i.g.)
DPP-4	Dipeptidyl peptidase-4
ELISA	Enzyme-linked immunosorbent assay
EM	Empagliflozin
GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide-1
GLUT	Glucose transporter
H&E	Hematoxylin and eosin
i.g.	Intragastric
i.p.	Intraperitoneal
MF	*Micromeria fruticosa* (L.) Druce subsp. *brachycalyx* P. H. Davis methanolic extract
NA	Nicotinamide
OGTT	Oral glucose tolerance test
PPAR-γ	Peroxisome proliferator-activated receptor gamma
SEM	Standard error of mean
SGLT	Sodium-glucose cotransporter
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TNF-α	Tumor necrosis factor-alpha
TP	*Teucrium polium* L. methanolic extract

1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycaemia which occurs due to defects in insulin secretion and/or insulin effect (Kharroubi and Darwish, 2015).

Medicinal plants for a very long time have traditionally been used to treat diabetes. (Elavarasi et al., 2013). Currently, the evidence on the efficacy of medicinal plant supplements' uses to prevent and treat T2DM is growing (Pivari et al., 2019).

Teucrium polium and *Micromeria fruticosa* subspecies both belong to the Lamiaceae family, and grow on the Mediterranean basin, and eastern Mediterranean coasts respectively where also lays Turkey (*Teucrium polium* also grows on arid and rocky areas of Europe, North Africa, and South West Asia) are known to be used by local residents in order to treat diabetes along with many other diseases (Özer et al., 2018; Abadian et al., 2016; Azap, 2016).

For more than 2000 years Teucrium species have been used as medicinal plants (Bagci et al., 2010). *Teucrium polium* due to its wide range of properties including its antiulcer, hypotensive, anorexigenic, antirheumatic, antibacterial, antifungal (Bahramikia et al., 2022), antihyperlipidemic, and antidiabetic properties have been used in traditional medicine (Albadr et al., 2022; Bahramikia et al., 2022). Besides, *Teucrium polium* has been stated to be one of the most commonly used medicinal plants in diabetes treatment in Turkey (Karaman and Elgin Cebe, 2016).

Germacrene D, t-cadinol, β-pinene, carvacrol, bicyclogermacrene, α-pinene, and limonene have been shown as the major compounds of the methanolic extracts of *Teucrium polium* L.'s aerial parts (Sharifi-Rad et al., 2022).

Micromeria fruticosa on the other hand, is one of the well-known subspecies of the genus Micromeria growing on the eastern coast of the Mediterranean. It has been documented in various reports that the traditional uses of this subspecies include curing different types of paralysis, nervous system disorders, headache, fever, treatment of urinary diseases, respiratory system illnesses; especially cough, and the treatment of diabetes (Azap, 2016).

Micromeria fruticosa L. have been shown to contain 215 phenolics and other compounds in total and over 180 phytochemicals, including 87 flavonoids, 41 phenolic acids, 16 terpenoids, 8 sulphate derivatives, 7 iridoids, and others (Abu-Reidah et al., 2019).

Our study aimed to enlighten the possible antidiabetic properties and to discover the possible mechanisms of the antidiabetic action of *Teucrium polium* L. and *Micromeria fruticosa* (L.) Druce subsp. *brachycalyx* P. H. Davis by evaluating the biochemical and histopathological analyses performed on rats with streptozotocin/nicotinamide-induced T2DM model.

2. Materials and methods

The materials and methods of plant extract preparation, experimental animal studies, biochemical analysis, histopathological analysis, and statistical analysis were given in Supplementary Materials.

3. Results

3.1. Dose determination study

At the 30th and 60th min of the dose determination study, the doses of 200 mg/kg for *Teucrium polium* L. methanolic extract (TP) and *Micromeria fruticosa* (L.) Druce subsp. *brachycalyx* P. H. Davis methanolic extract (MF) both decreased BGLs significantly compared to the control group (for TP - 200 mg/kg at 30th and 60th min $p < 0.01$, $p < 0.001$, for MF - 200 mg/kg at 30th and 60th min $p < 0.001$, $p < 0.01$ respectively) (Fig. 1). Based on the data obtained, the doses of "200 mg/kg" were selected for both medicinal plant extracts for the study.

3.2. Effect of MF, TP, and EM on body weights

Female adult Sprague Dawley rats with the same weight range (200–300 g) were used in the experimental protocol. The body weights of each animal in all groups were weekly measured during the experiment (Fig. 2a). C, DM + TP, DM + MF, and DM + EM groups maintained their body weights during the 3 weeks of the experiment, in body weights between the control, DM + TP, DM + MF, and DM + EM groups, a statistically significant difference was not obtained for 3 weeks of the treatment period. However, in the 3rd week compared to the control group, the body weight of the DM group decreased significantly ($p < 0.01$).

3.3. Effects of MF, TP, and EM on blood glucose levels

BGLs of rats were measured weekly during the experiment (Fig. 2b). After induction of T2DM, at the beginning of the treatments (week 0) no statistically significant difference was seen between experimental groups while the BGLs of all experimental groups were higher significantly in comparison to the control group (for all experimental groups $p < 0.001$).

DM group continued to have significantly high BGLs in comparison to the control group for 3 weeks of the experiment period (for each week $p < 0.001$). BGLs of DM + MF began to decrease significantly in comparison to the DM group at the end of the 2nd week of treatment ($p < 0.05$) and this decrease continued and was evident at the end of the treatment period (3rd week) ($p < 0.001$). Similarly, BGLs of DM + TP began to decrease significantly compared to DM at the end of the 2nd week ($p < 0.05$) and the decrease continued and was apparent at the end of the treatment period (3rd week) ($p < 0.001$).

DM + EM had the lowest BGLs compared to the DM group from the first week of the treatment ($p < 0.001$) to the end of the treatment period (for both the 2nd and 3rd weeks $p < 0.001$).

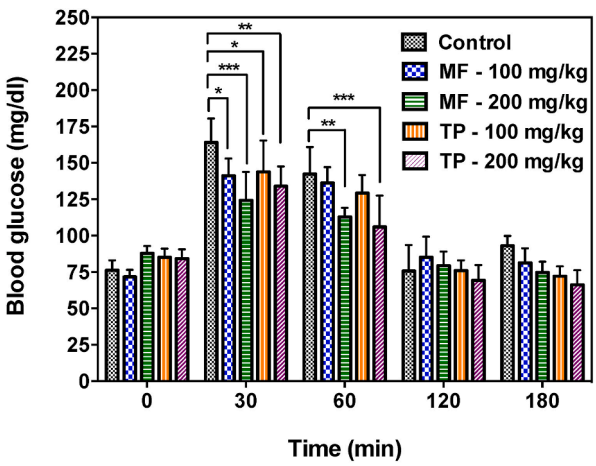

Fig. 1. BGLs (mg/dl) were obtained by OGTT in normoglycemic rats for dose determination. Values are presented as Mean ± SEM (n = 6). Significance at *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in comparison with the control group.

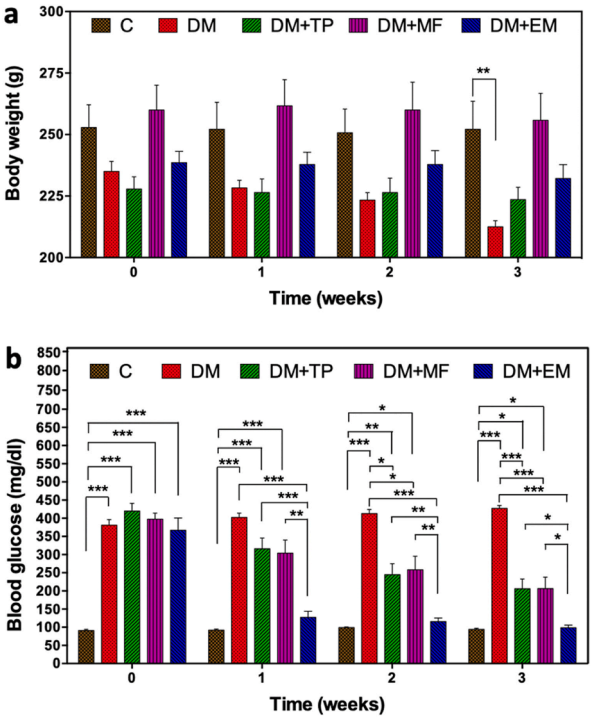

Fig. 2. (a) Body weight (g.) of rats for 3 weeks. Significance at **p < 0.01 in comparison to the control group. (b) BGLs (mg/dl) of rats for 3 weeks. Values are presented as Mean ± SEM (n = 8). Significance at *p < 0.05, **p < 0.01, ***p < 0.001 in comparison to each other.

3.4. The effects of MF, TP, and EM on OGTT

OGTT was performed following 12 h of fasting and with a glucose single dose (2.5 g/kg b. w.) as a solution while the treatments were carried out in treatment groups to evaluate the effect of TP and MF on the ability to regulate glucose metabolism, glucose-induced insulin secretion, and its mediated glycaemic changes in rats (Fig. 3a). At the beginning of the experiment, among all the groups no significant difference was seen. BGLs of the DM group in comparison to the control group were higher between the 30th-120th min of the test (at 30th min $p < 0.001$, at 60th min $p < 0.001$, at 90th min $p < 0.05$ and 120th min $p < 0.01$ respectively).

At the 30th min of OGTT, significantly higher BGLs for DM + TP in comparison to the control group were detected ($p < 0.001$). However, after the 30th min BGLs of DM + TP tend to decrease and at the 60th min came closer to the levels of the control group while still higher at the 60th and 90th minutes of the OGTT (for both minutes $p < 0.05$).

Similarly, at the 30th min of the test, significantly higher BGLs for DM + MF in comparison to the control group were detected ($p < 0.01$). However, compared to the control group, the BGLs of DM + MF started to decrease after the 30th min of the test but were still higher in comparison to the control group at the 60th min ($p < 0.001$) and after the 60th min of the test, at 90th min and by the end of the test no statistically significant difference in the BGLs of DM + MF group was found in comparison to the control group.

During the OGTT performed, between BGLs of DM + EM and the control group statistically no significant difference was obtained.

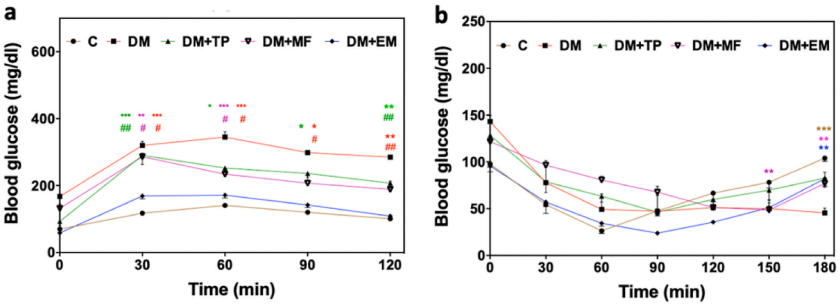

Fig. 3. (a) Blood glucose levels of rats on 0–120 min in oral glucose tolerance test (OGTT). Significance at *p < 0.05, **p < 0.01, ***p < 0.001 in comparison to the control group; #p < 0.05, ##p < 0.01, ###p < 0.001 in comparison to the DM + EM group. (b) Blood glucose (mg/dl) of rats on min 0-180 in insulin tolerance test (ITT). Values are presented as Mean ± SEM (n = 8). Significance at **p < 0.01 and ***p < 0.001 in comparison to DM.

3.5. Effect of MF, TP, and EM on ITT

In order to evaluate insulin sensitivity, ITT was applied to all rats after 12 h of fasting by an intraperitoneal injection of a single dose of 1 U/kg insulin (insulin lispro (Humolog®, Lilly)) while the treatments were carried out in treatment groups (Fig. 3b). The beginning of the test showed a significant decrease phase for BGLs in all groups (for the first 60 min in the control group, for the first 90 min in DM + EM, for the first 90 min in DM + TP, and the first 150 min in the DM + MF). The BGLs of the DM group, however, continued to decrease for 180 min (throughout the ITT), and this decrease in BGLs was hardly tolerated in the DM group. After this decrease phase, the BGLs for each group (except for the DM group) began to rise.

This decline in BGLs was quickly tolerated in the control and DM + EM groups after 60 min, and 90 min respectively.

While no significant difference was found among the groups according to BGLs in 120 min, BGLs of DM + MF showed a statistically significant increase in comparison to the DM group at the 150th min of the test ($p < 0.01$). At the end of the test (at 180th min), control, DM + MF, and DM + EM groups had a significant increase in BGLs compared to the DM group ($p < 0.001$, $p < 0.01$, $p < 0.01$ respectively).

3.6. The effects of MF, TP, and EM on GLP-1, GLUT-2, PPAR-γ, SGLT-2, insulin, and TNF-α levels

Compared to the control group statistically significant decreases were detected in GLP-1 levels in both pancreas and ileum tissues of the DM group (in pancreas tissue $p < 0.001$, in ileum tissue $p < 0.01$). In the ileum tissue, GLP-1 levels of DM + MF were significantly higher in comparison to the DM group ($p < 0.01$) (Fig. 4a).

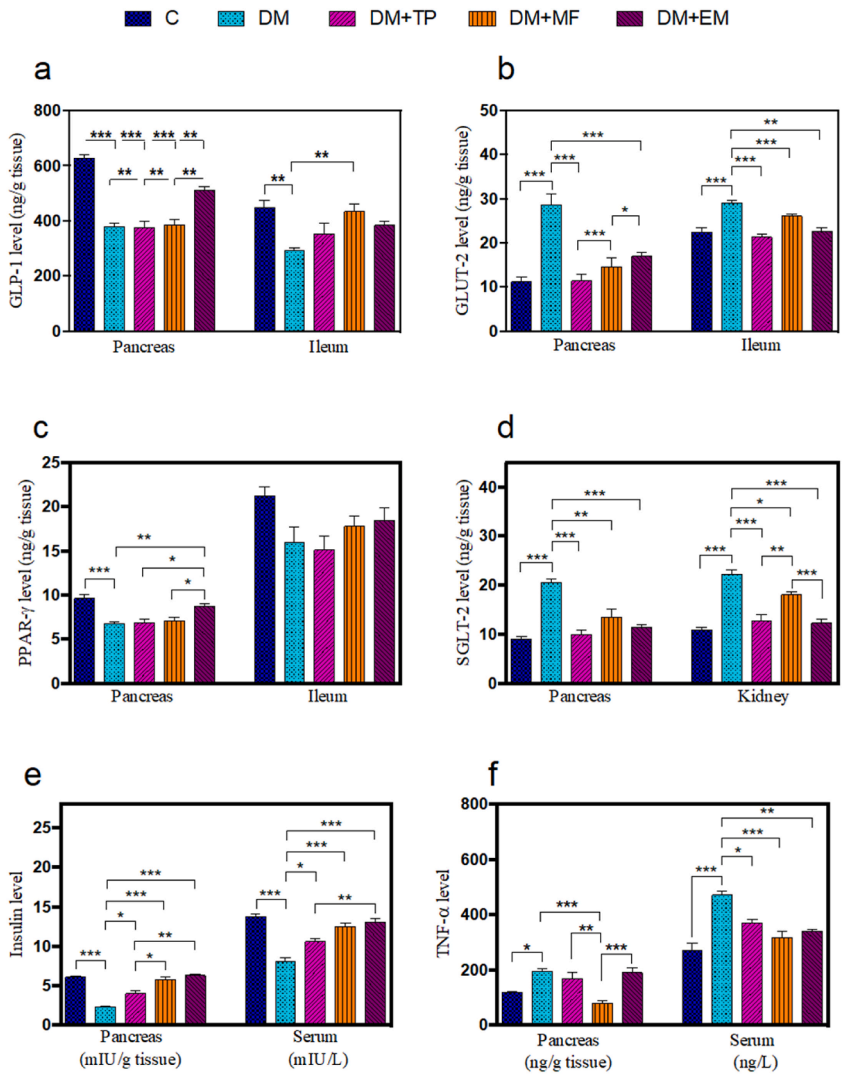

Fig. 4. Effects of TP, MF, and EM on (a) glucagon-like peptide-1 (GLP-1), (b) facilitated diffusion glucose transporter-2 (GLUT-2), (c) peroxisome proliferator-activated receptor gamma PPAR-γ, (d) sodium-glucose cotransporter-2 (SGLT-2), (e) insulin, and (f) tumor necrosis factor-alpha (TNF-α) levels. Values are expressed in Mean ± SEM (n = 8). One-way ANOVA was carried out followed by post-hoc Tukey multiple comparison test. Values are given statistically when *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ in comparison to each other.

GLUT-2 levels of DM increased significantly in the pancreas and ileum tissues compared to the control group (for both tissues $p < 0.001$). GLUT-2 levels in ileum tissues of DM + MF, DM + TP, and DM + EM significantly decreased compared to the DM group (for both DM + MF and DM + TP groups $p < 0.001$, and in ileum tissue for DM + EM $p < 0.01$). GLUT-2 levels in pancreas tissues significantly decreased in DM + TP and DM + EM groups compared to the DM group (for both treatments $p < 0.001$) (Fig. 4b).

Regarding the PPAR-γ levels in pancreas tissues, the DM group showed a significant decrease compared to the control group ($p < 0.001$) while in the DM + EM group, PPAR-γ levels significantly increased compared to the DM group ($p < 0.01$). No significant difference in PPAR-γ levels in ileum tissues was detected among the groups (Fig. 4c).

SGLT-2 levels in both pancreas and kidney tissues of the DM group were significantly higher compared to the control group (for both tissues $p < 0.001$). All treatment groups (DM + TP, DM + MF, and DM + EM) showed a significant decrease in SGLT-2 levels in both tissues in comparison to the DM group while the decrease in SGLT-2 levels in both tissues in DM + TP and DM + EM was more evident (for both groups and in both tissues $p < 0.001$) (Fig. 4d).

The levels of insulin in both serum and pancreas tissue were significantly low in the DM group compared to the control group (for both $p < 0.001$). On the other hand, insulin levels in all treatment groups were significantly higher in comparison to the DM group, and in DM + MF and DM + EM groups in insulin levels, the increases in both serum and pancreas were more apparent (for both groups and in both serum and pancreas tissues $p < 0.001$) (Fig. 4e).

In the DM group in comparison to the control group TNF-α levels increased in both pancreas tissue and (particularly) serum ($p < 0.05$, and $p < 0.001$ respectively). Serum TNF-α levels of all treatment groups (DM + EM, DM + TP, DM + MF) decreased in comparison to the DM group ($p < 0.01$, $p < 0.05$, $p < 0.001$ respectively). Decreases in the levels of TNF-α in both pancreas tissue and serum were more evident in the DM + MF group (for both $p < 0.001$) (Fig. 4f).

3.7. Effects of MF, TP, and EM on histopathology of liver, pancreas, and kidney

In the control group ($5A_1$), regular liver parenchyma with hepatocytes and sinusoids were observed. In the DM group ($5A_2$), hepatic cords' disorganization, degeneration in hepatocytes (arrowhead), and sinusoids' severe vascular congestion (arrow) were noted. In EM-, TP- and MF-treated DM groups ($5A_{3\text{-}5}$), hepatic cords' normal organization, and in hepatocytes decreased degeneration were observed.

In the control group ($5B_1$), normal pancreatic appearance was observed. In the DM group ($5B_2$), acinar necrosis (arrowheads), apoptotic cells (arrows), hypertrophic cells (*), and hemorrhage (white arrow) were noted. In EM-, TP-, and MF-treated DM groups ($5B_{3\text{-}5}$): mild acinar hypertrophy (*), apoptosis (arrow), and necrosis (arrowhead) were observed.

In the control group ($5C_1$), with Bowman's space, glomerular structures, and tubules, renal tissue of normal morphology was observed. In the DM group ($5C_2$): glomerular structures' degeneration and Bowman's space dilatation (*), proximal tubules' degeneration (arrow), and vascular congestion (v) were observed. In EM-, TP- and MF-treated DM groups ($5C_{3\text{-}5}$), glomerular structures and Bowman's space of normal morphology (*) and proximal tubule's mild degeneration (arrow) in most regions were noted. H&E staining scale bars: 100 μm.

In the control group light microscopic evaluation, liver parenchyma of a regular morphology with intact hepatocytes and sinusoids was detected (Fig. $5A_1$). Hepatic cords were disorganized in the DM group (Fig. $5A_2$). Moreover, degenerated hepatocytes with pycnotic nuclei, prominently activated Kupffer cells, and sinusoids with severe vascular congestion were observed. EM, TP, and MF-treated DM groups showed hepatic cords with normal organization. Although all of the treatments ameliorated these morphological

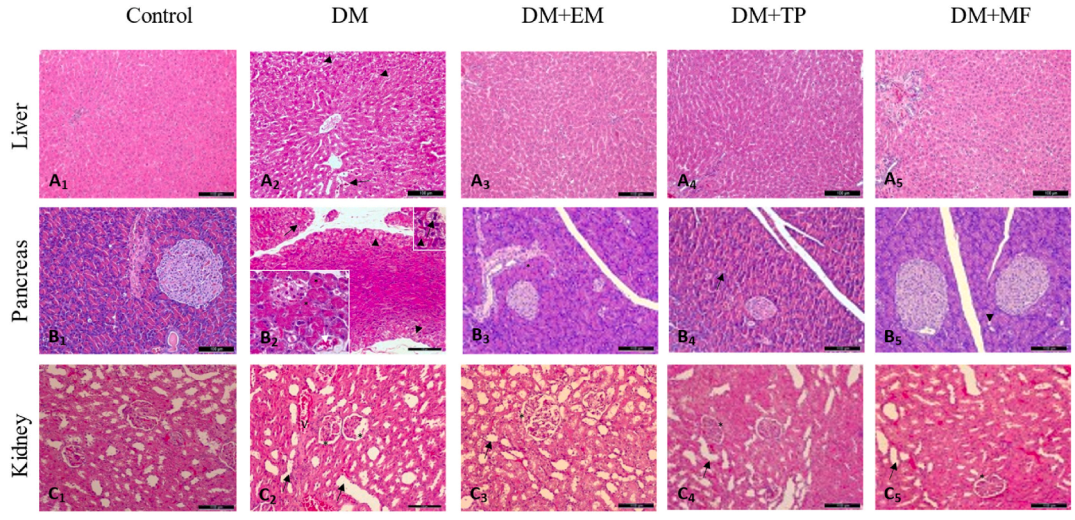

Fig. 5. Liver, pancreas, and kidney cortex representative photomicrographs in experimental groups (50 μm and 100 μm scaled).

degenerations (Fig. 5A_{3-5}), the MF-treated DM group showed more activated Kupffer cells than other treatments and the EM-treated DM group showed more regular morphology like the control group than other treatments.

In the control group, with their apical acidophilic cytoplasm and basal nuclei pancreatic acinar cells and regular islet morphology were observed (Fig. 5B_1). In the DM group, besides evident acinar cell hypertrophy and apoptosis/necrosis, increased degenerated endocrine cells were also observed (Fig. 5B_2). Histopathologic changes in the pancreas were moderately improved in the EM-, TP- and MF-treated DM groups (Fig. 5B_{3-5}). Treatment groups and the positive control group showed mild acinar hypertrophy, apoptosis, and necrosis. EM-treated DM group revealed quite regular morphology with an increased pancreatic regeneration in comparison to other treatment groups.

Although in the control group, a renal parenchyma of normal morphology with regular glomeruli and tubules was observed (Fig. 5C_1), the DM group presented Bowman's space dilatation, degenerations in proximal tubules and glomerular structures, and moderate vascular congestion (Fig. 5C_2). However, the kidneys of EM-, TP- and MF-treated DM groups showed a better morphology of glomeruli, proximal tubules, and Bowman's spaces (Fig. 5C_{3-5}).

4. Discussion

T2DM is a major and emerging global health problem and although pharmacotherapy for T2DM is available, it still has limitations (such as poor adherence to medicines, and the side effects of the medicines). Indeed, many patients with diabetes have been stated to use complementary therapies (ranging from 17% in the UK to 72% in the US), and “herbal medicines” have been reported to be among the most commonly used complementary therapy for diabetes (such as in Saudi Arabia, and Mexico %68, and %62 of patients with diabetes have been reported to use herbal medicines respectively) (Willcox et al., 2021). Since plants are stated to provide one of the best options for the search for desired safe and effective medications for DM, many plants have been investigated for their antidiabetic effects, and mechanisms of antidiabetic action (Patel et al., 2012; Ozkum et al., 2013).

Although the antihyperglycemic effects of *Teucrium polium* L. were shown in various studies (Vahidi et al., 2010; Mahjoub et al., 2012), there are no published studies in the literature investigating the antidiabetic effects and the mechanisms of antidiabetic action of *Micromeria fruticosa* (L.) Druce subsp. *brachycalyx* P. H. Davis. In our study, at the end of the 3 weeks of the treatment, compared to the DM group, both TP and MF treatments showed remarkable decreases in the BGLs of type 2 diabetic rats. However, at the end of the three weeks of treatment period, empagliflozin was more effective in decreasing BGLs of type 2 diabetic rats than TP and MF.

To further investigate and support the antidiabetic effects of TP and MF treatments we also performed OGTT and ITT, and in these tests, these treatments showed better BGL profiles than the DM group. Empagliflozin treatment also showed better BGL profiles than the DM group in both OGTT and ITT, especially in OGTT the BGL profile obtained with EM treatment was close to that of the control group. These findings indicated all treatments (more apparently EM) could improve glucose tolerance and impair insulin tolerance. Insulin resistance, one of the principal pathophysiologies of T2DM (American Diabetes Association, 2022) forms an important risk factor in the progress of glucose tolerance, and DM, and these results we obtained showed that the treatments with TP, MF, and EM could diminish insulin resistance. These findings together demonstrated these medicinal extracts could be beneficial for the treatment of T2DM.

Characteristic weight loss resulting from the catabolism of tissue proteins and muscle wasting in diabetic rats has been correlated with DM induction (Çam et al., 2017). In our study, we also found that for 3 weeks of treatments, TP and MF (and also empagliflozin) treatment groups maintained their body weights while the body weights of the DM group in 3rd week, with also the effects of fasting periods applied for the OGTT and ITT performed in the 3rd week of the experiment, significantly decreased compared to the control group. Significant body weight loss in type 2 diabetic rats with the same body weight range at the end of the 3rd week of the treatment period was also found in a similar previous study (Çam et al., 2017). Apart from this finding in the DM group, no statistically significant finding was obtained among the groups, and no body weight loss was observed in the treatment groups in the treatment period. Taken together these findings indicated that MF and TP could have antidiabetic effects, and we further researched the underlying mechanisms of the antidiabetic actions of these medicinal plants in T2DM.

In T2DM progressive loss of adequate pancreatic β cell mass, β cell function, and insulin secretion occur (Cerf, 2013). Supporting these data; the results of our study showed that insulin levels in both pancreas and serum in the DM group decreased significantly in comparison to the control group. Our study results also showed that in all treatment groups insulin levels in both serum and pancreas increased significantly compared to the DM group with more apparent increases obtained in MF and EM-treated groups. In addition, the histopathological findings of our study showed that while pancreatic β cell destruction was observed in the DM group, moderately improved histopathological changes in the pancreas were seen in the treatment groups. These histopathological findings were correlated with increased insulin levels in MF, TP, and EM-treated groups compared to the DM group and demonstrated that enhancing insulin levels could be one of the underlying mechanisms of antidiabetic action of MF and TP treatments. Insulinotropic properties of methanolic extract and ethanol/water extract of *Teucrium polium* L. have been documented in previous studies (Yazdanparast et al., 2005; Mirghazanfari et al., 2010). Our findings are consistent with and support the literature data, and it is of interest that, in our study compared to the DM group, MF treatment showed more apparent increases in insulin levels in pancreas tissues and serum than TP (the methanolic extract of *Teucrium polium* L.; a medicinal plant which has demonstrated insulinotropic properties with previous studies) treatment, suggesting that MF could also have insulinotropic properties which can be investigated with further studies.

Increased levels of various pro-inflammatory cytokines contribute to the development of insulin resistance and pathogenesis of T2DM, and TNF-α is one of the most important pro-inflammatory cytokines involved in these processes (Akash et al., 2018). In our study, TNF-α levels of the DM group increased in the pancreas and (particularly) in serum in comparison to the control group supporting these data. We found TP, MF, and EM treatments reduced serum TNF-α levels compared to the DM group, moreover, the reduction

in MF treatment in both serum and pancreas was remarkable. These results are consistent with reduced TNF-α levels in serum obtained with *Teucrium polium* L. in an animal study of hypercholesterolemia which was stated to contribute to the antioxidant, and anti-inflammatory effects of *Teucrium polium* L. (Amraei et al., 2018). There have been no previous studies on MF's effects on TNF-α levels, and as discussed previously, in our study with MF treatment a more evident reduction in TNF-α levels in both serum and pancreas than TP treatment compared to the DM group was detected. Since *Teucrium polium* L. is a medicinal plant that was previously shown to reduce TNF-α levels, taken together these results indicate that MF could also have antioxidant and anti-inflammatory effects which could contribute to its antidiabetic effects, and can be investigated with further studies.

GLUT-2 is a glucose transporter isoform that is expressed in the liver, kidney, and pancreatic β-cells, and also in the central nervous system, in neurons, astrocytes, and tanycytes, and plays an important role in glucose homeostasis including glucose sensing in pancreas and liver, glucose uptake in the liver, in pancreatic β-cells glucose-stimulated insulin secretion, transepithelial glucose transport in intestine and glucose reabsorption in kidneys (Thorens, 2015). Diabetic animal models demonstrated increased GLUT-2 expression in the ileum and liver tissues (Burant et al., 1994). In our study, while in the DM group GLUT-2 levels in the ileum tissues significantly increased compared to the control group, in the ileum tissues of TP and MF-treated diabetic rats statistically significant decreases in GLUT-2 levels were obtained indicating that one of the antidiabetic mechanisms of action of these medicinal plants could be due to reducing GLUT-2 levels in ileum tissues.

SGLT-2 is another glucose transporter located mainly in the kidneys' renal proximal convoluted tubules, and less in the small intestine (ileum), is responsible for almost 90% of renal glucose uptake, and inhibiting SGLT-2 has become one of the targets for T2DM treatment (Cangoz et al., 2013). Various studies have reported increased SGLT-2 expressions in renal tissues of diabetic animals (Osorio et al., 2012; Tabatabai et al., 2009). In recent years, SGLT-2 inhibitors which stimulate the excretion of glucose via urine by kidneys began to be used in the treatment of T2DM (Shin et al., 2016). In our study, SGLT-2 levels in the kidneys of the DM group demonstrated a remarkable increase in comparison to the control group. While in all treatment groups, significant decreases in SGLT-2 levels in kidneys were determined compared to the DM group, the decrease in TP-treated and EM-treated diabetic groups was remarkable. Empagliflozin is an SGLT-2 inhibitor that received approval from The US Food and Drug Administration (FDA) and European Medicines Agency (EMA) in 2014, and is being used in T2DM treatment (Inzucchi et al., 2015). Since TP, and EM (a SGLT-2 inhibitor) treatments both showed a similar decrease in SGLT-2 levels in kidney tissues compared to the DM group, we can confidently say that the inhibition of SGLT-2 in kidneys may lead to the antidiabetic effects of TP. Besides, if not as apparent as the TP or EM treatment, in the kidney tissues of type 2 diabetic rats MF treatment also significantly reduced SGLT-2 levels compared to the DM group, and we can also say reducing SGLT-2 levels in kidneys could be one of the mechanisms of antidiabetic action of MF treatment.

GLP-1 is an intestinal hormone that has been accepted as an ideal target for diabetes treatment since it has several effects on glucose metabolism including enhancing the production and secretion of glucose-dependent insulin, and in peripheral tissues increasing the uptake of glucose and synthesis of glycogen, decreasing the secretion of glucagon, delaying gastric emptying and increasing satiety, and over the years many GLP-1 agonists were found and took place in diabetes treatment (Sheahan et al., 2020). In diabetic rats decreased levels of GLP-1 in the ileum and pancreas were shown (Kartinah et al., 2019). In our study in the ileum tissues of the DM group GLP-1 levels significantly decreased in comparison to the control group. Besides, in comparison to the DM group, in the MF-treated group, a significant increase in the GLP-1 levels in the ileum tissues was detected. As we discussed previously, the levels of insulin in both pancreas and serum of the MF-treated group were found significantly increased compared to the DM group, and since GLP-1 enhances glucose-dependent insulin production and secretion, taken together these results suggest that MF treatment increases GLP-1 levels in the ileum and this may contribute to its effect on increased insulin levels in MF treatment we found in our study, and may also be one of the mechanisms of antidiabetic action of MF.

PPAR-γ is one of the peroxisome proliferator-activated receptors (PPARs) and its activation leads to several metabolic functions including insulin sensitization and enhancing glucose metabolism (Tyagi et al., 2011). PPAR-γ agonists (thiazolidinediones) which activate PPAR-γ

Receptors are being used in T2DM treatment (Azhar 2010; Okur et al., 2017). Downregulations in PPAR-γ protein expressions were shown in diabetic rat models (Ajayi et al., 2021; Li et al., 2019; Assaei et al., 2016; Kanie et al., 2003). Our results showed in comparison to the control group PPAR-γ levels in the pancreas tissue of the DM group significantly decreased, while neither MF nor TP treatment showed any significant difference in comparison to the DM group, the EM-treated group showed a statistically significant increase in PPAR-γ levels in pancreas tissue in comparison to the DM group. In addition, in the ileum tissues, no significant difference was detected among groups for PPAR-γ levels. These results indicate that the antidiabetic effects of neither TP nor MF could be associated with PPAR-γ levels in the pancreas or ileum.

STZ, the diabetogenic agent used in the study causes damage in the pancreas, liver, and kidneys of the experimental animals, and this is the main reason for the damages observed in the pancreas, liver, and kidneys of the diabetic experimental animals in the study (Fig. 5) (Eleazu et al., 2013). Although there have been reports of hepatotoxicity and nephrotoxicity associated with *Teucrium polium* L. (Baradaran et al., 2013; Shahraki et al., 2007; Zal et al., 2001), our study's histopathological results showed that after 3 weeks of the treatment period, while disorganized hepatic cords, degenerated hepatocytes with pycnotic nuclei, and sinusoids with severe vascular congestion and prominent activated Kupffer cells were observed in the DM group, in EM, TP, and MF-treated diabetic groups, the hepatic cords showed normal organization and all of the treatments ameliorated these morphological degenerations seen in the DM group. In addition, regarding the histopathological analysis of the kidney tissues, although dilated Bowman's space, degenerated proximal tubules and glomerular structures, and moderate vascular congestion were observed in the DM group, in the kidneys of EM-, TP- and MF-treated diabetic groups, glomeruli, proximal tubules, and Bowman's spaces showed a better morphology. In our study it was also demonstrated that while in the histopathological analysis of the pancreas of the DM group evident acinar cell hypertrophy

and apoptosis/necrosis and degenerated endocrine cells were observed, histopathologic changes in the pancreas were moderately improved in the EM-, TP- and MF-treated diabetic groups.

In addition to our favourable histopathological findings, it has been noted in a current review that the active compounds that contribute to the antidiabetic properties of *Teucrium polium* extract were not likely to be the same active compounds associated with the reported hepatotoxicity. Even if so, with drug development techniques, and knowledge about the associated pathological mechanisms involved, it is possible to improve and prevent such side effects, and *Teucrium polium* can be evaluated as a potential source of drug for T2DM (Albadr et al., 2022).

In the present study, the antidiabetic effects and the mechanisms of antidiabetic action of TP and MF were investigated over insulin, TNF-α, GLUT-2, SGLT-2, GLP-1, and PPAR-γ levels, and in certain tissues as detailly given above in a rat model of STZ/NA-induced T2DM, and the presented findings showed that these medicinal plants could be drug sources and/or guide for future drug researches and also could be beneficial for the treatment of T2DM. The changes in the proteins we examined should be supported with different studies, and further studies including the investigation of other structures (e.g., enzymes, glucose transporters, etc.), and conditions (e.g., insulin resistance) associated with the pathophysiology of T2DM, and safety studies (e.g., long-term toxicology studies, etc.) should be conducted on these medicinal plants.

5. Conclusion

In the present study, we investigated the antidiabetic effects, and the mechanisms of antidiabetic action of TP and MF over insulin, TNF-α, GLUT-2, SGLT-2, GLP-1, and PPAR-γ levels in a rat model of STZ/NA-induced T2DM.

Our results indicated that both TP and MF have potential "antidiabetic effects", and while the antidiabetic mechanisms of action of TP could be due to **(i)** an increase in the levels of insulin in both pancreas and serum, **(ii)** a decrease in the levels of TNF-α levels in serum, **(iii)** decrease in the levels of GLUT-2 levels in ileum tissue, and **(iv)** decrease in the levels of SGLT-2 levels in kidneys; the antidiabetic mechanisms of action of MF could be due to **(i)** an increase in levels of insulin in both pancreas and serum, **(ii)** a decrease in the levels of TNF-α in both serum and pancreas tissue, **(iii)** a decrease in the levels of GLUT-2 in ileum tissue, **(iv)** a decrease in the levels of SGLT-2 in kidneys and **(v)** an increase in the levels of GLP-1 levels in ileum tissue in STZ/NA-induced type 2 diabetic rats. Although we didn't observe any signs of toxicity in the histopathological analysis of the pancreas, liver, and kidney, since there have been reports of hepatotoxicity and nephrotoxicity associated with *Teucrium polium* L., this should be taken into consideration. In light of these findings, TP and MF might be potential drug sources and/or guides for future drug investigations for T2DM treatment. Further studies are required to investigate the efficacy and safety of *Teucrium polium* L. and *Micromeria fruticosa* (L.) Druce subsp. *brachycalyx* P. H. Davis in the treatment of T2DM.

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CRediT authorship contribution statement

Sinan Sermet: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Visualization, Project administration, Writing – review & editing, Supervision. **Muhammet Emin Cam:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Visualization, Project administration, Writing – review & editing, Supervision. **Ayse Nur Hazar-Yavuz:** Investigation, Resources, Data curation. **Turgut Taskin:** Conceptualization, Investigation, Resources. **Gul Sinemcan Kabatas:** Investigation, Resources, Formal analysis, Visualization. **Yusufhan Yazir:** Investigation, Resources, Formal analysis, Visualization. **Levent Kabasakal:** Writing – review & editing. **Hatice Kubra Elcioglu:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

Authors all declare that there is no existing conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.obmed.2023.100517.

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