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RESEARCH

Gastroprotective role of *Myrtus communis* in a dual-condition model combining diabetes and postmenopausal rats: comparable outcomes to estrogen therapy

Diyabet ve postmenopozal sıçanları birleştiren ikili durum modelinde *Myrtus communis*'in gastroprotektif rolü: östrojen tedavisi ile karşılaştırılabilir sonuçlar

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Abstract

Purpose: Hormonal changes during menopause, especially the decline in estrogen and progesterone levels, can impair gastrointestinal function by slowing digestion. This study aimed to evaluate the protective effects of *Myrtus communis* subsp. *communis* (MC) extract on gastric tissue in a postmenopausal diabetic rat model and to compare its efficacy with estrogen (E2) treatment.

Materials and Methods: Female Sprague-Dawley rats were divided into six groups: control (C); ovariectomy (OVX); diabetes (D); ovariectomy and diabetes (OVX+D); ovariectomy, diabetes and estrogen (OVX+D+E2); ovariectomy, diabetes and MC (OVX+D+MC). OVX groups had bilateral ovariectomy utilizing the double dorsolateral method. After a seven-day recovery, diabetes was induced using streptozotocin. OVX+D+E2 and OVX+D+MC groups were treated with 10 µg/kg s.c. E2 and 100 mg/kg p.o. MC extract, respectively, for four weeks. Stomach tissues were analyzed for biochemical parameters.

Results: The OVX, D, and OVX+D showed significant reductions in antioxidant enzyme activities and glutathione levels, and increases in oxidative stress markers and lipid peroxidation compared to the C group. E2 and MC treatments effectively mitigated these alterations, with MC showing comparable or superior effects to E2 in many parameters. MC treatment significantly improved blood glucose levels (~60% reduction in the OVX+D), stomach Na^+/K^+-ATPase activity (~110% increase in the OVX+D), and glycoprotein component levels.

Conclusion: MC extract exhibits potent antioxidant and gastroprotective effects in postmenopausal diabetic rats,

Öz

Amaç: Menopoz sırasındaki hormonal değişiklikler, özellikle östrojen ve progesteron seviyelerindeki düşüş, sindirimi yavaşlatarak gastrointestinal işlevi bozabilir. Bu çalışma, *Myrtus communis* subsp. *communis* (MC) özütünün postmenopozal diyabetik sıçan modelinde gastrik doku üzerindeki koruyucu etkilerini değerlendirmeyi ve etkinliğini östrojen (E2) tedavisiyle karşılaştırmayı amaçlamaktadır.

Gereç ve Yöntem: Dişi Sprague-Dawley sıçanları altı gruba ayrıldı: kontrol (C); overektomi (OVX); diyabet (D); overektomi ve diyabet (OVX+D); overektomi, diyabet ve östrojen (OVX+D+E2); overektomi, diyabet ve MC (OVX+D+MC). OVX grupları, çift dorsolateral yöntemi kullanarak bilateral overektomiye tabi tutuldu. Yedi günlük iyileşme süresinin ardından, streptozotosin kullanılarak diyabet oluşturuldu. OVX+D+E2 ve OVX+D+MC gruplarına sırasıyla 10 µg/kg s.c. E2 ve 100 mg/kg p.o. MC ekstresi dört hafta süreyle uygulandı. Mide dokusu biyokimyasal parametreler açısından analiz edildi.

Bulgular: OVX, D ve OVX+D, C grubuna kıyasla antioksidan enzim aktivitelerinde ve glutatyon seviyelerinde önemli azalmalar ve oksidatif stres belirteçlerinde ve lipid peroksidasyonunda artışlar gösterdi. E2 ve MC tedavileri bu olumsuz değişiklikleri etkili bir şekilde hafifletti ve MC, birçok parametrede E2 ile karşılaştırılabilir veya daha üstün etkiler gösterdi. MC tedavisi kan glikoz seviyelerini (OVX+D'de ~%60 azalma), ve mide dokusundaki Na^+/K^+-ATPaz aktivitesini (OVX+D'de ~%110 artış) ve glikoproteinlerin seviyelerini önemli ölçüde iyileştirdi.

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comparable to E2 treatment. These findings highlight its potential as a natural therapeutic agent for managing menopause- and diabetes-associated gastric complications.

Keywords: Myrtle, oxidative stress, ovariectomy, postmenopausal diabetes, stomach.

Sonuç: MC özütü, E2 tedavisiyle kıyaslanabilecek şekilde, menopoz sonrası diyabetli sıçanlarda güçlü antioksidan ve gastroprotektif etkiler göstermektedir. Bu bulgular, MC'nin menopoz ve diyabetle ilişkili gastrik komplikasyonları yönetmek için doğal bir terapötik ajan olarak potansiyelini vurgulamaktadır.

Anahtar kelimeler: Menopoz sonrası diyabet, Mersin bitkisi, mide, oksidatif stres, overektomi.

INTRODUCTION

Myrtle (*Myrtus communis* subsp. *communis*, Myrtaceae, MC), a medicinal plant native to the Mediterranean region of Türkiye, has been widely used in traditional medicine for its diverse therapeutic properties. This aromatic plant is known for its insecticidal, antidiabetic, antioxidant, anti-inflammatory, and antimicrobial effects. Various parts of the plant, including the fruits, branches, and leaves, have traditionally been employed to treat conditions such as digestive disorders, peptic ulcers, and skin diseases[1,2]. Recent *in vitro* and *in vivo* studies have shown that it has a wider range of pharmacological and therapeutic effects, which include the treatment of urinary tract infections, rheumatism, diabetes, anxiety, insomnia, hypertension and menstruation difficulties[3]. Terpenoids, flavonoids, phenolics, tannins, and polyunsaturated fatty acids are among the key volatile phytochemicals commonly found in myrtle. The anti-inflammatory and antioxidant effects of myrtle extracts are primarily attributed to their phenolic compounds, such as caffeic, ellagic, and gallic acids. Notably, the leaves of the plant contain higher concentrations of antioxidant substances than other parts, making them the most pharmacologically valuable component. While the myrtle fruit and leaves are rich in tannins, the stems are characterized by high levels of flavonoids, particularly galloyl derivatives of catechin[3]. Due to these biologically active substances, it is successful in treating digestive disorders[4]. The astringent, tonic and antibacterial properties of its leaves explain its use for healing wounds or curing diseases of the digestive system and urinary tract[5]. These properties suggest a potential role for myrtle in addressing oxidative stress-related conditions, especially in the gastrointestinal system.

The gastrointestinal tract plays a key role in coordinating nutritional responses and regulating hormonal interactions involved in digestion and glycemic control[6]. However, this complex and intricate system is especially vulnerable to complications arising from chronic conditions such as diabetes. It is well known that diabetes mellitus plays a significant role in the development of oxidative damage in various tissues, including the gastrointestinal system, particularly the gastric mucosa. This damage is primarily mediated by excessive production of reactive oxygen species (ROS) and insufficient antioxidant defense, which together lead to structural and functional impairments of the stomach lining[6]. In addition to oxidative stress, patients with long-standing diabetes mellitus often experience acute elevations in serum glucose concentration, which adversely affect gastric motility. This leads to delayed gastric emptying, a condition known as gastroparesis. Consequently, diabetic patients often experience gastrointestinal discomfort, poor nutrient absorption, and reduced quality of life[7]. Given these challenges, the antioxidant properties of myrtle could help mitigate the oxidative stress and inflammation associated with diabetic gastrointestinal complications.

In addition to diabetes, hormonal changes during menopause also introduce additional risks to the gastrointestinal system. Declines in estrogen (E2) and progesterone levels during menopause can slow down the digestive process, increase water reabsorption in the intestines, and may lead to symptoms such as constipation, gas, and bloating. Postmenopausal women are also more susceptible to age-related gastric mucosal atrophy[8]. The situation becomes even more concerning when menopause and diabetes coexist, as declining E2 levels in postmenopausal women heighten their susceptibility to oxidative stress. E2 deficiency not only accelerates cellular damage but also exacerbates metabolic disorders such as diabetes. This creates a synergistic environment of heightened oxidative stress, rendering the gastric system particularly vulnerable[9]. A dual-condition model combining diabetes and postmenopausal states, hormonal changes such as reduced E2 amplify oxidative stress, while diabetes further increases the risk of oxidative damage. This model mimics conditions in postmenopausal women

with diabetes. In such cases, the phenolic components of myrtle may provide therapeutic benefits by reducing oxidative damage and improving gastrointestinal health.

Although previous studies have investigated the antioxidant and anti-inflammatory effects of *Myrtus communis* in various models, there is limited evidence regarding its protective role in gastrointestinal complications arising from the coexistence of menopause and diabetes. This study aims to investigate whether the MC's antioxidant properties can mitigate diabetes-induced oxidative stress and restore impaired metabolic functions in the dual-condition model combining diabetes and postmenopausal animal models. This research contributes to the literature by providing a comprehensive biochemical analysis of how MC modulates oxidative stress, glycoprotein accumulation, and antioxidant enzyme activities in stomach tissue. We hypothesize that MC extract exerts protective effects against gastric damage induced by the combined impact of menopause and diabetes by enhancing antioxidant defense mechanisms, reducing oxidative stress, and regulating glycoprotein levels.

MATERIALS AND METHODS

Plant material and preparation of MC extract

Plant samples from the Turgutlu district of Manisa, in Türkiye (38.5° N, 27.7° E) were collected in 2010 and identified by Dr. Gizem Bulut, a botanist at the Faculty of Pharmacy, Marmara University. Voucher specimens were deposited in the Herbarium of the same faculty (Herbarium Protocol No: 13006). Ethanolic MC extract was prepared according to the method previously described in Kadıoğlu Yaman et al[2]. Fresh leaves of *Myrtus communis* (100 g) were air-dried in the shade at ambient temperature. The dried leaves were then ground into a fine powder and extracted with 96% ethanol using a Soxhlet extractor. The resulting extract was filtered and concentrated under reduced pressure at 40°C using a rotary evaporator. The final dried powder was stored in a dark container at +4°C in a refrigerator until required for activity assays.

Animals and experimental design

All animals were obtained from the Marmara University Experimental Animal Implementation and Research Centre. The experimental protocols were approved by the Marmara University Local Ethics Committee for Animal Experiments (Protocol number: 42.2023.mar, dated 15.08.2023), and conducted in accordance with institutional guidelines and the ARRIVE reporting standards.

Experiments were conducted on female Sprague-Dawley rats, ten weeks old and weighing 200–250 g. They were given a typical pellet diet and maintained in a room with controlled temperature and lighting on a 12-hour/12-hour period. Rats were randomly divided into the following six groups: sham-operated control (C, n=8); ovariectomy (OVX, n=10); diabetes (D, n=10); ovariectomy and diabetes (OVX+D, n=10); ovariectomy, diabetes and estrogen (OVX+D+E2, n=5); ovariectomy, diabetes and *Myrtus communis* subsp. *communis* (OVX+D+MC, n=10). MC extract (100 mg/kg p.o.) was dissolved in distilled water. Streptozotocin (45 mg/kg i.p.) was freshly prepared in citrate buffer (pH 4.5)[2]. Rats were anesthetized with ketamine (100 mg/kg i.p.) and chlorpromazine (0.7 mg/kg i.p.), and bilateral ovariectomy was performed using the double dorsolateral method in the OVX, OVX+D, OVX+D+MC, and OVX+D+E2 groups[10]. After a seven-day recovery period following ovariectomy, diabetes was induced with 45 mg/kg STZ (i.p). Sham-operated rats received citrate buffer, and their ovaries were identified but not removed. Forty-eight hours after STZ injections, OVX+D+E2 and OVX+D+MC groups received 10μg/kg E2 (s.c.) and 100 mg/kg MC extract (p.o.), respectively, for four weeks.

Isolation of stomach and preparation of tissue homogenate

At the end of the experiment, blood samples were collected to measure blood glucose levels, and animals were euthanized. Following decapitation, stomach tissues were obtained and stored at -20°C for later analysis. The entire stomach was used for biochemical analysis. The tissues were homogenized in 0.9% NaCl solution to prepare a 10% (w/v) homogenate for biochemical evaluation at +4°C.

Biochemical analysis

Blood glucose levels were measured at both the beginning and end of the experiments. Reduced glutathione (GSH) levels were determined according to Beutler's method[11], which quantifies GSH based on its reaction with 5,5'-dithiobis-(2-nitrobenzoic acid), producing a yellow compound measured at 412 nm. Lipid peroxidation (LPO) was assessed by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reaction under acidic conditions, producing a pink chromophore detected at 532 nm, as described by Ledwozyw et al[12]. Glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT), superoxide dismutase (SOD) activities were assayed according to the medhod's of Beutler, Wendel, Habig and Jakoby, Aebi, Mylroie et al., respectively[13–17]. These enzyme activities were monitored kinetically. Lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PDH), and carbonic anhydrase (CA) activities were determined using the methods of Bais and Philcox, Beutler, and Verpoorte et al., respectively, and were also measured spectrophotometrically[18–20]. Sodium potassium ATPase (Na^+/K^+-ATPase) and xanthine oxidase (XO) activities and protein carbonyl (PC) contents were assessed using the methods of Ridderstap and Bonting, Corte and Stirpe, and Levine et al., respectively[21–23]. Reactive oxygen species (ROS), nitric oxide (NO) and hydroxyproline (OH-proline) levels were measured using the methods of Zhang et al., Miranda et al., and Reddy and Enwemeka, respectively[24–26]. DNA content was determined by the diphenylamine method described by Burton[27]. Total protein in the stomach homogenates was measured using the Lowry method[28]. All biochemical results were expressed per protein amount, and analyses were performed manually in accordance with the referenced methods.

Glycoprotein levels determination

Sialic acid levels were measured according to the method of Lorenz et al.[29]. Hexose, hexosamine, and fucose levels were analyzed by Winzler and by Dische and Shettles[30,31].

Dr. Duke database search for bioactive compounds

The bioactive compounds of *Myrtus communis* leaf were identified using Dr. Duke's Phytochemical and Ethnobotanical Database (https://phytochem.nal.usda.gov) using the keywords "*Myrtus communis (Myrtaceae)*".

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software Inc., USA). Variables with homogeneous distribution — including blood glucose, GSH, LPO, PC, ROS, NO, OH-proline, DNA content, sialic acid, hexose, hexosamine, fucose levels, and the enzymatic activities of GR, GPx, GST, CAT, SOD, LDH, G6PDH, CA, Na^+/K^+-ATPase, and XO — were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine pairwise group differences. Comparisons were made across six experimental groups (C, OVX, D, OVX+D, OVX+D+E2, OVX+D+MC) for each biochemical and glycoprotein parameters. Data were presented as mean ± standard error of the mean (SEM). Results were considered statistically significant at $p < 0.05$. Pearson's correlation analysis was calculated to find the direction and strength of the correlation between the biochemical parameters in the stomach tissue.

RESULTS

Biochemical parameters show *Myrtus* mitigates the blood glucose levels, Na^+/K^+-ATPase activity, and glycoprotein component levels in the stomach tissue of postmenopausal diabetic rats

Fasting blood glucose levels were found to be significantly increased ($p < 0.0001$) in D and OVX+D, while glucose levels in E2 or MC-treated rats were shown to be significantly decreased. The OVX+D+MC group glucose level was closer to control but significantly ($p < 0.001$) lower than that of the OVX+D+E2 group (Figure 1).

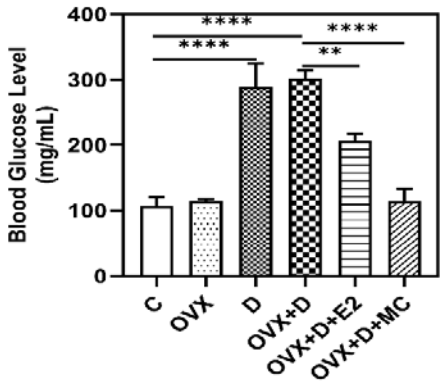

Figure 1. Blood glucose levels of the control and experimental groups of rats.

Data are presented as mean ± SEM. $^{**}p < 0.01$ and $^{****}p < 0.0001$. C: Sham-operated control; OVX: ovariectomy; D: diabetes; OVX+D: ovariectomy and diabetes; OVX+D+E2: ovariectomy, diabetes and estrogen; OVX+D+MC: ovariectomy, diabetes, and *Myrtus communis* subsp. *communis*.

Figure 2 depicts changes in stomach GSH and LPO levels. Compared to the C, a decrease in the GSH level was found in the OVX, D and OVX+D ($p < 0.001$ and $p < 0.001$). However, E2 or MC reversed these defects and greatly improved ($p < 0.01$) the GSH levels afterwards in E2 or MC-treated OVX+D. When compared to C, the increase in the LPO levels was found in the OVX, D and OVX+D ($p < 0.0001$ and $p < 0.001$). Similarly, E2 or MC remarkably dropped LPO levels in E2 ($p < 0.0001$) or MC-treated OVX+D.

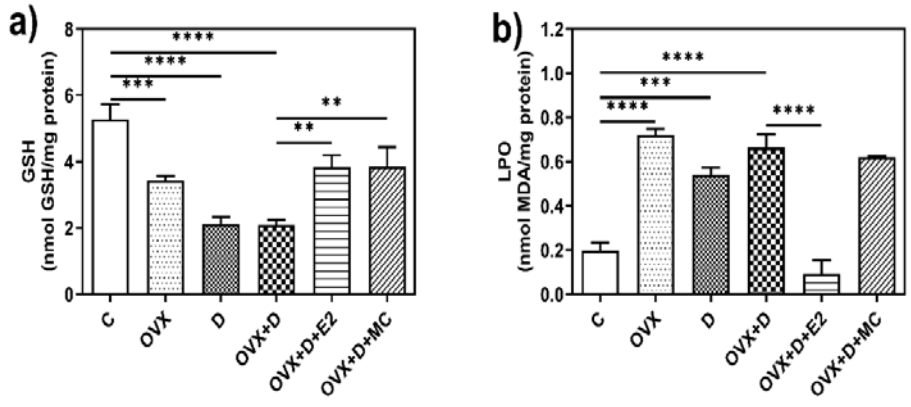

Figure 2. Stomach a) GSH and b) LPO levels of control and experimental groups of rats.

Data are presented as mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. C: Sham-operated control; OVX: ovariectomy; D: diabetes; OVX+D: ovariectomy and diabetes; OVX+D+E2: ovariectomy, diabetes and estrogen; OVX+D+MC: ovariectomy, diabetes, and *Myrtus communis* subsp. *communis*. GSH: reduced glutathione; LPO: lipid peroxidation.

The enzymatic activities of GR, GST, GPx, CAT, and SOD in the stomach are shown in Figure 3. Stomach GR, GST, GPx, CAT and SOD activities were dramatically reduced in the OVX, D and OVX+D ($p < 0.0001$ and $p < 0.001$). E2 or MC treatments significantly restored the activity of these enzymes in the OVX+D ($p < 0.0001$).

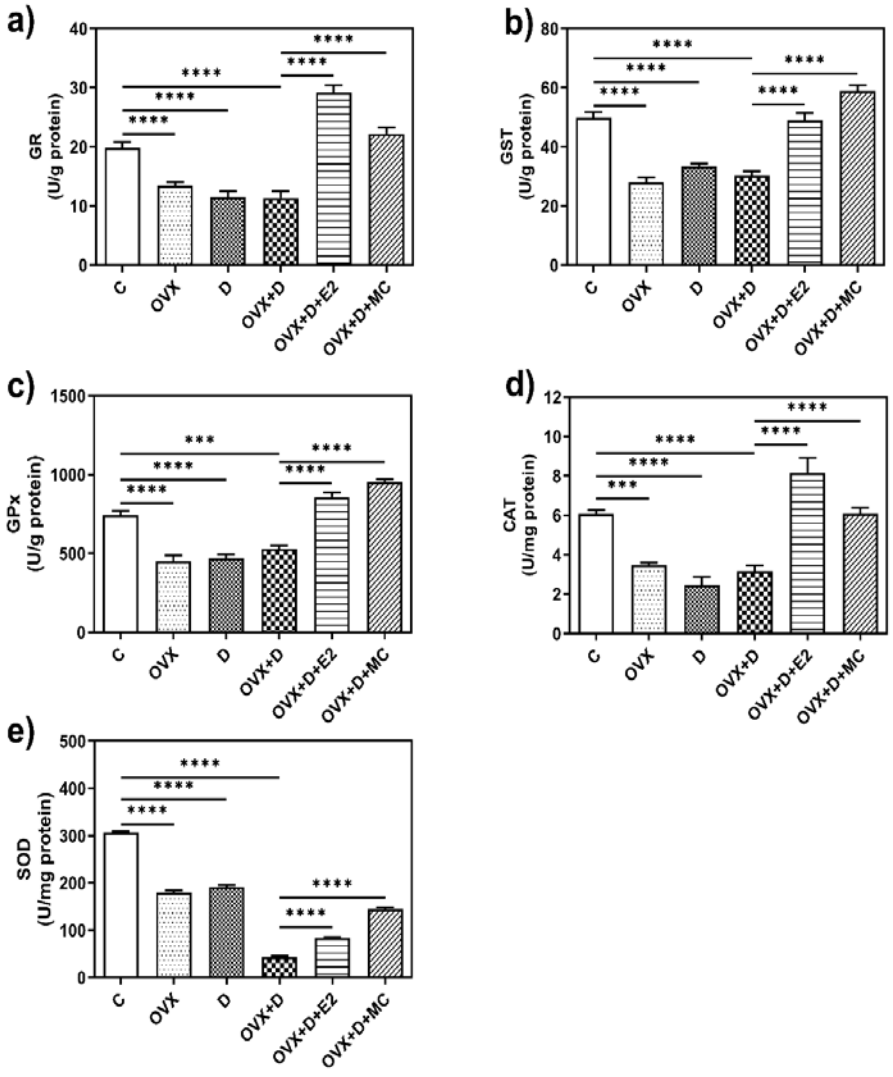

Figure 3. Stomach a) GR, b) GST, c) GPx, d) CAT and e) SOD activities of control and experimental groups of rats.

Data are presented as mean ± SEM. ***$p < 0.001$ and ****$p < 0.0001$. C: Sham-operated control; OVX: ovariectomy; D: diabetes; OVX+D: ovariectomy and diabetes; OVX+D+E2: ovariectomy, diabetes and estrogen; OVX+D+MC: ovariectomy, diabetes, and *Myrtus communis* subsp. *communis*. CAT: catalase; GPx: glutathione peroxidase; GR: glutathione reductase: GST, glutathione-S-transferase: SOD, superoxide dismutase.

Figure 4 shows the changes in stomach LDH, G6PDH, and CA activities of C and experimental rats. The activity of LDH ($p < 0.0001$) was significantly increased, while G6PDH and CA activities were significantly decreased ($p < 0.01$, $p < 0.0001$ and $p < 0.001$) in the OVX, D and OVX+D. E2 or MC treatments significantly reversed these changes in LDH activity ($p < 0.0001$) and G6PDH and CA activities in the OVX+D ($p < 0.0001$).

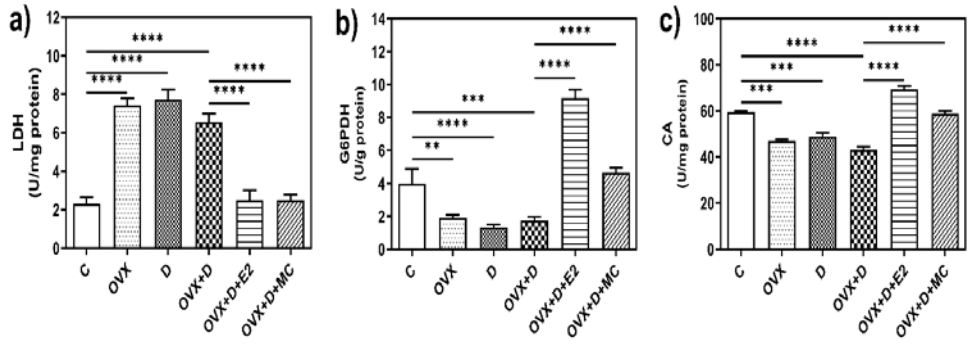

Figure 4. Stomach a) LDH, b) G6PDH and c) CA activities of control and experimental groups of rats.

Data are presented as mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. C: Sham-operated control; OVX: ovariectomy; D: diabetes; OVX+D: ovariectomy and diabetes; OVX+D+E2: ovariectomy, diabetes and estrogen; OVX+D+MC: ovariectomy, diabetes, and *Myrtus communis* subsp. *communis*. CA: carbonic anhydrase; G6PDH: glucose-6-phosphate dehydrogenase; LDH: lactate dehydrogenase.

The Na^+/K^+-ATPase and XO activities and PC levels in the stomach tissue are shown in Figure 5. In comparison to C, the activities of Na^+/K^+-ATPase were significantly decreased ($p < 0.0001$), whereas XO activities and PC levels were significantly increased ($p < 0.0001$ and $p < 0.001$) in the OVX, D and OVX+D. Both E2 or MC reversed these changes in Na^+/K^+-ATPase ($p < 0.05$, $p < 0.01$) and XO activities and PC levels ($p < 0.0001$, $p < 0.05$ and $p < 0.01$) in the stomach of OVX+D.

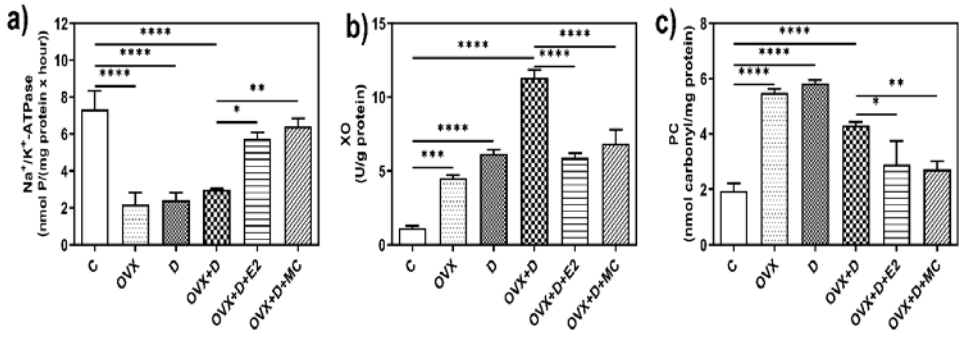

Figure 5. Stomach a) Na^+/K^+-ATPase, b) XO activities, and c) PC levels of control and experimental groups of rats.

Data are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. C: Sham-operated control; OVX: ovariectomy; D: diabetes; OVX+D: ovariectomy and diabetes; OVX+D+E2: ovariectomy, diabetes and estrogen; OVX+D+MC: ovariectomy, diabetes, and *Myrtus communis* subsp. *communis*. Na^+/K^+-ATPase: sodium potassium ATPase; PC: protein carbonyl; XO: xanthine oxidase.

Changes in stomach ROS, NO, and OH-proline levels and DNA content of C and experimental rats are depicted in Figure 6. The ROS, NO, OH-proline levels were significantly increased ($p < 0.0001$, and $p < 0.01$), whereas DNA contents were significantly decreased in the OVX, D and OVX+D compared to the C group ($p < 0.0001$). Administration of E2 or MC reversed these changes in ROS, NO, DNA contents ($p < 0.0001$) and OH-proline levels in the stomach of OVX+D.

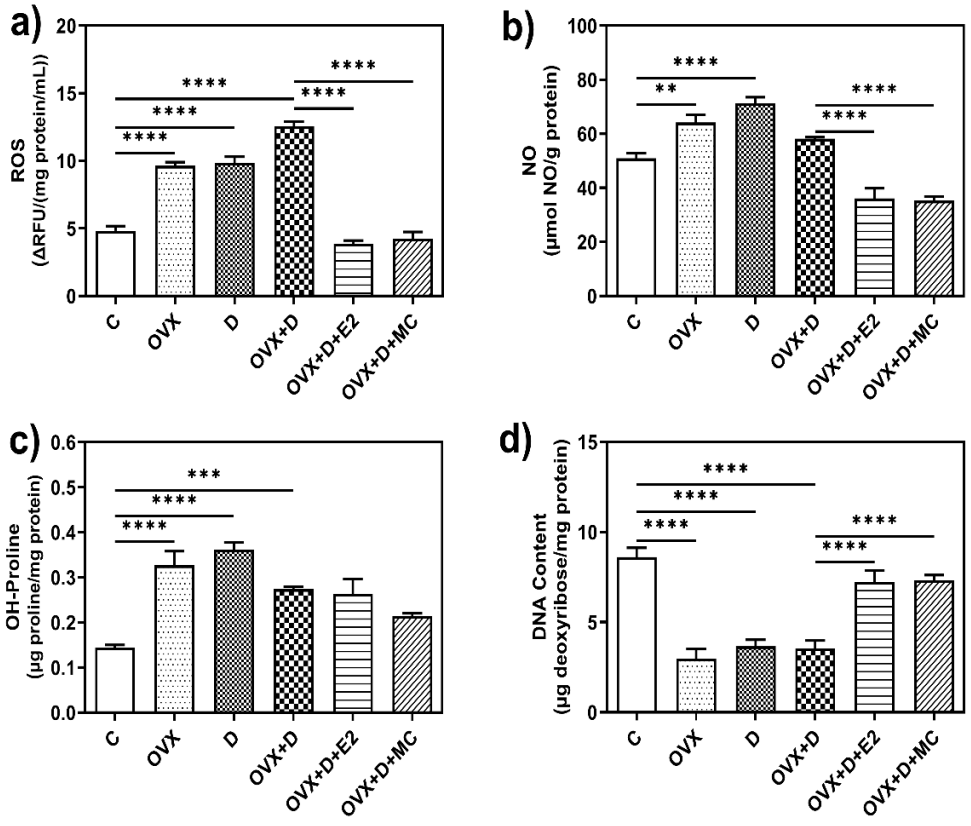

Figure 6. Stomach a) ROS, b) NO, c) OH-proline and d) DNA content levels of control and experimental groups of rats.

Data are presented as mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. C: Sham-operated control; OVX: ovariectomy; D: diabetes; OVX+D: ovariectomy and diabetes; OVX+D+E2: ovariectomy, diabetes and estrogen; OVX+D+MC: ovariectomy, diabetes, and *Myrtus communis* subsp. *communis*. DNA: deoxyribonucleic acid; NO: nitric oxide; ROS: reactive oxygen species.

Figure 7 depicts the differences in the levels of tissue glycoprotein components in C and experimental rats. The levels of glycoproteins containing sialic acid, hexose, hexosamine, and fucose were significantly increased the OVX, D and OVX+D compared to C ($p < 0.0001, p < 0.01$). The E2 or MC intake produced a reduction in the level of glycoprotein components in the stomach of OVX+D ($p < 0.001$ and $p < 0.0001$).

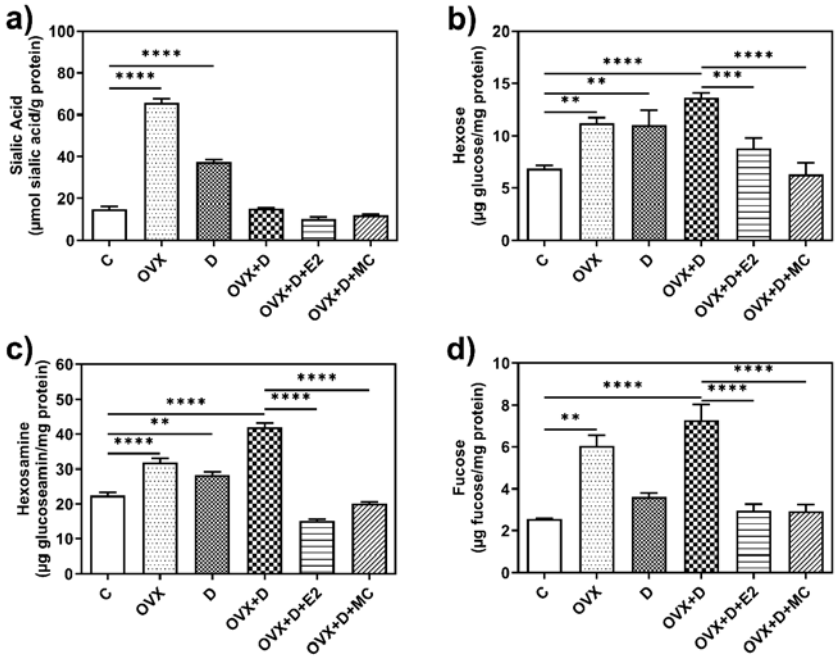

Figure 7. Stomach a) sialic acid, b) hexose, c) hexosamine and d) fucose levels of control and experimental groups of rats.

C: Sham-operated control; OVX: ovariectomy; D: diabetes; OVX+D: ovariectomy and diabetes; OVX+D+E2: ovariectomy, diabetes and estrogen; OVX+D+MC: ovariectomy, diabetes, and *Myrtus communis* subsp. *communis*. Data are presented as mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

According to Dr. Duke's Phytochemical and Ethnobotanical Databases, the biocomponents present in *Myrtus* are summarized in Table 1. The "activity count" refers to the number of known biological or pharmacological actions attributed to a compound. "Low parts per million" and "high parts per million" represent the range of concentrations of a compound within a plant. Based on this data, *Myrtus* leaves are particularly abundant in terpenes.

Table 1. Bioactive compounds in *Myrtus* leaf according to Dr. Duke's Phytochemical and Ethnobotanical Database.

Chemical Name	Activity Count	Low Parts Per Million	High Parts Per Million
1,8-Cineole	67	135	2250
Alpha-pinene	28	150	1900
Alpha-terpineol	23	20	600
Geraniol	35	1	75
Geranyl-acetate	5	8	250
Limonene	60	40	585
Myrtenol	2	3	250
Myrtenyl-acetate	1	40	1000
Myrtocommulon-a	0	not available	not available
Myrtocommulon-b	0	not available	not available
Myrtol	0	not available	not available

The Pearson correlation coefficient is a statistical tool used to assess the relationship between two continuous variables. Values close to +1 indicate a perfect positive correlation, meaning that an increase in one variable corresponds directly to an increase in the other, while values near -1 signify a perfect negative correlation, where an increase in one variable corresponds to a decrease in the other. Pearson's correlation analysis of all the biochemical parameters revealed both significant positive and negative correlations. GSH levels were significantly positively correlated with GST, CAT, CA and Na^+/K^+-ATPase activities and DNA content, while they significantly negatively correlated with LDH and XO activities, PC, ROS, OH-proline, hexose and hexosamine levels (Figure 8). The activities of antioxidant enzymes (GR, GST, GPx, CAT and SOD), as well as G6PDH, CA and Na^+/K^+-ATPase, showed positive correlation with each others and significant negative correlation with LDH activity, LPO, PC, ROS, NO, OH-proline and glycoprotein levels. Correlation analysis of glycoprotein levels was significantly positively correlated with LPO, PC, ROS, NO, OH-proline levels, and with LDH and XO activities.

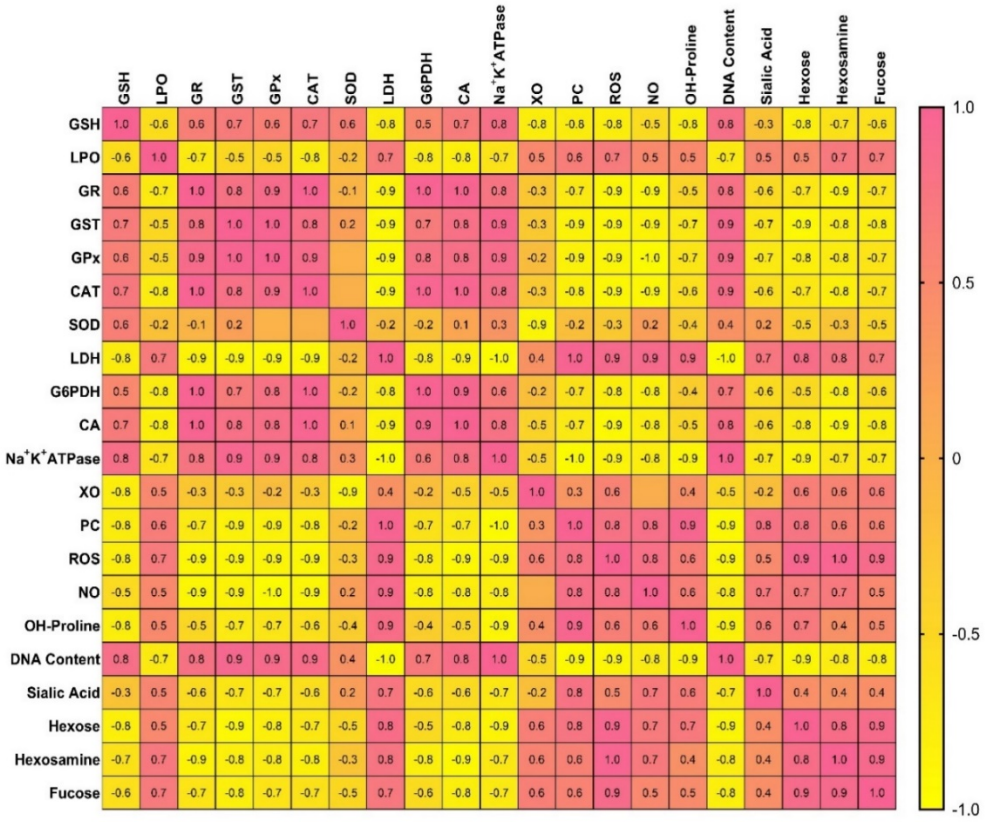

Figure 8. Heatmap diagram illustrating the correlation matrix among various biochemical parameters in stomach tissue.

CAT: catalase; DNA: deoxyribonucleic acid; GPx: glutathione peroxidase; GR: glutathione reductase; GSH: reduced glutathione; GST: glutathione-S-transferase; LPO: lipid peroxidation; Na^+/K^+-ATPase: sodium potassium ATPase; NO: nitric oxide; PC: protein carbonyl; ROS: reactive oxygen species; SOD: superoxide dismutase; XO: xanthine oxidase.

DISCUSSION

Estrogens play a key role in regulating metabolic processes such as body mass index, fat distribution, and energy balance[32]. During menopause, decreased estrogen levels are linked to increased oxidative stress, inflammation, delayed gastric emptying, and impaired gut barrier function, raising the risk of gastrointestinal complications, highlighting estrogen's protective role in metabolic and gut health[8]. This study investigated the effects of MC extract on gastric tissue damage caused by the combined challenges of menopause and diabetes, using an OVX rat model. The OVX rats modelled the postmenopausal state, while diabetes was induced to assess its independent and combined effects on gastric tissue. Our findings confirm that both conditions significantly increase oxidative stress and impair gastric antioxidant defense systems.

Biochemical markers of oxidative stress in gastric tissue, such as LPO level, an indicator of membrane damage, and GSH level, an antioxidant compound, provided valuable insights into the extent of cellular damage. Increased LPO and decreased GSH levels observed in the OVX, D and OVX+D groups are consistent with in previous studies, such as Samy et al., who reported similar reductions in GSH levels and SOD activities in ovariectomized mice[33]. The D group showed significant oxidative stress compared to the C group, suggesting that diabetes alone can severely disrupt gastric homeostasis, independent of estrogen deficiency. Notably, when combined with OVX, oxidative stress markers were further exacerbated, indicating a synergistic effect between estrogen deficiency and diabetes. MC extract treatment successfully reversed these oxidative markers, highlighting its potent antioxidant activity. These effects are likely due to its rich content of flavonoids and flavonols[34]. According to Dr. Duke's Phytochemical and Ethnobotanical Databases, *Myrtus* leaves are particularly abundant in terpenes (Table 1), many of which exhibit strong antioxidant properties that help mitigate oxidative stress. This aligns with Jabri et al., who demonstrated that myrtle inhibits LPO and increases both non-enzymatic antioxidant levels and antioxidant enzymes activity in the stomach and duodenal mucosa[35].

Previous studies have shown that OVX-induced osteoporosis in femoral tissues leads to a significant decrease in antioxidant enzyme activities (i.e., GR, GST, GPx and CAT)[36]. In our study, similar decreases were observed in stomach tissue. In particular, the D group significantly impaired these enzymatic antioxidant defense systems, which aligns with previous findings that chronic hyperglycemia contributes to ROS overproduction and antioxidant depletion. However, treatment with E2 or MC extract successfully restored these enzyme activities, demonstrating their potential in combating oxidative damage.

CAT activity is a critical and helpful parameter to evaluate the redox status of biological samples, as it plays a crucial role in neutralizing H_2O_2. A decrease in CAT activity is indicative of oxidative stress. In a study, the CAT activity was found to be decreased in the liver tissues of OVX rats compared to the C group[37]. Consistently, our results showed that OVX decreased CAT activity by nearly 57%. Diabetes also contributed significantly to the suppression of CAT activity, further supporting the idea that hyperglycemia intensifies oxidative damage. However, E2 or MC treatment significantly increased CAT activity, with MC treatment showing a 193% improvement, closely following the 258% increase observed with E2 treatment. This suggests that MC may offer a comparable, if not superior, protective effect against oxidative damage in gastric tissues compared to E2.

The decrease in GSH may be attributed to its rapid consumption in scavenging excess ROS. The decrease in SOD activity further reflects the compromised enzymatic antioxidant defense, contributing to an increase in ROS formation. E2 deficiency during menopause enhances oxidative stress by increasing ROS production[38]. In this study, markedly elevated ROS levels were detected in the gastric tissues of rats in the OVX, D and OVX+D groups. Treatment with MC extract significantly alleviated ROS levels. The role of diabetes in gastric tissue damage is further evidenced by its impact on LDH activity. After a muscular injury, LDH activity increases quickly when the plasma membrane is compromised. A study showed that E2 treatment alleviated contusion-induced myoinjury in OVX mice by reducing LDH activity[39]. In another study, LDH activity was increased by oxidative stress-induced liver damage in ovariectomized mice[40]. Similarly, diabetes-induced oxidative stress led to an increase in LDH activity in the D and OVX+D groups, suggesting significant cellular membrane disruption due to hyperglycemia. Our findings showed that LDH activity in the OVX, D, and OVX+D groups

increased nearly 3.2, 3.4, and 2.9-fold compared to C, respectively. Both E2 and MC treatments effectively reduced LDH activity in OVX+D, indicating their protective effects on cellular integrity.

Diabetes also directly impacts mitochondrial function and energy metabolism, as demonstrated by the significant decrease in G6PDH activity observed in the D group. G6PDH converts $NADP^+$ to NADPH and provides the turnover of GSH, thus increasing the organism's defense against ROS. Oliveira et al. reported that G6PDH activity in the livers of mice with OVX was reduced by 33% compared to the control group and suggested that OVX-induced increase in oxidative stress[41]. Barker reported that three days of treatment with estradiol increased the activity of uterine G6PDH eightfold in ovariectomized rats[42]. Consistent with these findings, our results revealed a significant decrease in G6PDH activity in OVX and diabetic rats, while treatment with E2 or MC restored G6PDH activity to control levels. This suggests that MC, similar to E2, contributes to the enhancement of antioxidant defenses through G6PDH activation.

The decreased activity of Na^+/K^+-ATPase is accepted as an indicator of membrane damage, and a reduction in activity has been associated with oxidative stress in many studies. In their review, Obradovic et al., while suggesting that Na^+/K^+-ATPase enzyme activity is decreased in obesity and cardiovascular diseases, also stated that the enzyme activity is regulated by estradiol[43]. Most hormones, including insulin, E2, aldosterone, thyroid hormone, catecholamines, and glucocorticoids, increase Na^+/K^+-ATPase activity or the production of its subunits, while also activating certain signaling cascades[44]. Our results are consistent with these studies. Diabetes and OVX caused a significant decrease in Na^+/K^+-ATPase activity, while E2 or MC treatment reversed the enzyme activity.

XO activity was reduced by E2 supplementation in the aorta of OVX compared to control female rats[45]. *Myrtus communis* has antioxidant properties (e.g., myricetin). These properties inhibit XO activity, lipid peroxidation, and scavenge the free radicals. A small amount of myricetin derivatives reduces XO activity[46]. We found that MC administration decreased XO activity in the OVX+D group, which is consistent with the literature.

Protein carbonylation is a protein oxidation that disrupts the structure and function of proteins and is a biomarker in aging and disease[47]. Some studies showed that PC levels increased in the diabetic group when compared to the control group[48,49]. Hepatic biomarkers induced by CCl_4 have been reported to significantly inhibit the increase of plasma lipid and PC levels in rats after treatment with MC essential oil[50]. In our study, we found that E2 or MC therapies decreased PC levels significantly in the stomach of OVX+D.

In addition to causing cellular damage, oxidative stress may also disrupt mitochondrial or DNA repair systems. The increase of ROS, mutation, misreplication, apoptosis, or cancer formation causes DNA damage. Also, ovariectomy causes DNA damage as it creates E2 deficiency. In a study, the DNA content in the femoral diaphysis was significantly reduced as a result of ovariectomy[51]. In the study, we found that DNA levels decreased in the OVX, D, and OVX+D groups. Administration of E2 or MC reversed DNA levels in the stomach of OVX+D groups.

NO regulates diverse gastrointestinal, respiratory and genitourinary tract processes. When this molecule is present in low concentrations, it may protect cells, but when it is present in higher concentrations, it can function as a cytotoxin that promotes tumor angiogenesis and growth[52]. NO is overproduced in response to hyperglycemia, which leads to oxidative stress and tissue damage. Also, there is a relationship between pro-inflammatory activities and high NO levels[53]. Our study showed elevated NO levels in the OVX, D, and OVX+D groups, which were significantly reduced by E2 or MC treatments. This suggests that MC may mitigate the pro-inflammatory effects of excessive NO production, as previously reported in other studies[54].

Hydroxyproline is a main component of collagen[55]. Its level was increased in D, OVX, and OVX+D groups. Glycoprotein components such as hexose, hexosamine, fucose, and sialic acid were significantly increased in the D and OVX+D groups. Serum glucose levels above normal and insulin insufficiency may cause an increase in the production of glycoprotein components. *In vivo* hypoglycemic, hypolipidemic, and anti-oxidative stress properties of *Myrtus communis* L. fruit hydroalcoholic extract were studied by Tas et al. in diabetic rats, and it was discovered that enhanced insulin secretion led to lower blood glucose and lipid levels[56]. In a study, increased levels of glycoproteins were reported in gastric damage due to administering valproate to

experimental animals[57]. They found that glycoprotein components were significantly increased in STZ-induced diabetic rats' lungs and stomachs[58,59]. In a study by Ertik et al., glycoprotein levels were observed to be increased in the diabetic group compared to the control[60]. Similarly, we found that the levels of glycoproteins significantly increased in the OVX, D and OVX+D compared to C group. Treatment with E2 or MC effectively reduced these levels, suggesting that MC may modulate glycoprotein synthesis and contribute to the repair of gastric tissues.

The A-ring phenolic hydroxyl group in E2 works as an efficient electron donor, a free radical scavenger, and halts the lipoperoxidation process[61]. Moreover, E2 protects women from oxidative stress by promoting the development of antioxidants such as GPx and manganese superoxide dismutase (MnSOD)[62]. Nevertheless, hormone replacement therapy is not widely accepted, mostly because it is contraindicated in certain people, has poor compliance, is disliked for its adverse effects, has a risk of myocardial infarction, and has potential long-term cancer concerns. Therefore, the use of myrtle extract is of great importance in the treatment of the effects caused by ovariectomy. Myrtle exhibits antimutagenic and antioxidant activities, and the absence of mutagenicity in its purified compounds support its potential as a phytopharmaceutical agent.

Our findings unequivocally demonstrate that this unbalanced redox state is the result of antioxidant enzymes' decreased activity brought on by OVX and diabetes. While diabetes independently contributes to oxidative stress and metabolic dysregulation, OVX further exacerbates these effects by weakening the body's defense mechanisms. Our study showed that both E2 and myrtle extract exert protective effects against disruptions in glucose metabolism, lipid profile, and the antioxidant system induced by the combined impact of diabetes and estrogen deficiency in rats. Pearson correlation analysis further supported these findings, revealing a negative correlation between antioxidants/antioxidant enzyme levels and oxidants/glycoprotein levels.

In conclusion, *Myrtus communis* extract possesses potent antioxidant and protective effects on gastric tissue, particularly in the context of oxidative stress induced by menopause and diabetes. These findings suggest that *Myrtus communis* extract could serve as a natural and effective alternative to hormone replacement therapy. Nonetheless, limitations of the study include the lack of human studies and limited dose-response evaluations. Future research would focus on long-term safety, histopathological validation, and potential interactions with standard therapies, particularly in populations affected by both menopause and diabetes.

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Ethical Approval: All animals were obtained from the Marmara University Experimental Animal Implementation and Research Centre. The experimental protocols were approved by the Marmara University Local Ethics Committee for Animal Experiments (Protocol number: 42.2023.mar, dated 15.08.2023), and conducted in accordance with institutional guidelines and the ARRIVE reporting standards.
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