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# Antioxidant effect of chamomile tea on the salivary glands of streptozotocin-induced diabetic rats

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**Abstract:** This study aimed to analyze oxidative stress and the activity of antioxidant enzymes in the salivary glands of streptozotocin (STZ)-induced diabetic rats with *ad libitum* consumption of chamomile tea in substitution of water for 21 days. Rats were divided in two control groups (untreated control and treated control) and two diabetic groups (untreated diabetic and treated diabetic). Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) activities, total antioxidant status (TAS), and malondialdehyde (MDA) concentrations were determined. The chemical composition of the chamomile essential oil revealed 39 compounds, accounting for 93.5% of the total oils. The polyphenolic profile of the tea showed the presence of apigenin, luteolin, umbelliferone, and esculetin. SOD, GPx, CAT, and TAS levels were lower in the parotid (PA) diabetic glands, but treatment increased their concentration in both the submandibular (SM) and PA diabetic salivary glands. Increased MDA levels were observed in the PA diabetic glands, which were decreased by the consumption of chamomile tea with a reduction in hyperglycemia compared to that in untreated diabetic rats. However, the SM diabetic glands showed no difference in the MDA content. The consumption of chamomile tea prevented oxidative stress in the PA glands of diabetic rats, exhibiting hypoglycemic and antioxidant effects. Thus, chamomile tea could be a potential candidate for preventing oral complications in diabetes mellitus.

**Keywords:** Antioxidants; Diabetes Mellitus; Salivary Glands; Chamomile; Oxidative Stress.

## Introduction

Diabetes mellitus (DM) is a metabolic disorder of multiple etiologies, characterized by chronic hyperglycemia and changes in the metabolism of carbohydrates, fatty acids, and proteins, resulting from defects in insulin secretion, its action, or both.[1] It is well known that hyperglycemia plays an important role in the pathogenesis of diabetes in humans and in experimental models. Chronic hyperglycemia results in morphological changes and dysfunction of various organs, including the salivary glands. Xerostomia is a symptom frequently reported in diabetic patients.[2,3] Prolonged exposure to high glucose concentrations leads to oxidative stress, which reduces the capacity of the endogenous antioxidant defense

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system and induces the formation of reactive oxygen species (ROS) by several molecular mechanisms.[4,5] Oxidative stress is a condition resulting from an imbalance between ROS production and elimination.[6]

Mitochondria are the main source of ROS under normal conditions.[7] Mitochondrial glucose oxidation generates ATP, and a small amount of superoxide anion is converted to other ROS.[8] ROS targets include proteins, lipids, and DNA. Nevertheless, there is an antioxidant defense system that protects the organism from the oxidative effects of ROS. However, excessive production of ROS in diabetes leads to protein glycation, which can produce structural modifications in enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT).[9] This results in a decrease in antioxidant defense and an increase in malondialdehyde (MDA),[10-12] an important marker of oxidative stress. This process can occur in a variety of tissues, including salivary glands.[13] Recent studies have demonstrated that the pathogenesis of salivary gland and oral cavity complications in diabetes is closely associated with oxidative stress.[14] Such evidence suggests that antioxidants are a promising additional treatment for DM.[15]

Epidemiological data have demonstrated that chamomile consumption is beneficial in DM because of its antioxidant effect and biological properties.[16] In addition, previous studies have shown that treatment with chamomile tea or chamomile tea components led to a reduction in glycemia.[16-19] Cemek et al. indicated that treatment with different doses of ethanolic extract of chamomile reduced postprandial hyperglycemia and MDA content, and increased the antioxidant system activity in streptozotocin (STZ)-induced diabetic rats.[18] These effects have been attributed to two classes of compounds: sesquiterpenes and phenols. A possible beneficial effect of chamomile tea is related to polyphenolic flavonoids such as apigenin, which was shown to decrease serum glucose concentrations in diabetic rats.[20] In addition, the effect of chamomile tea is related to the presence of polyphenols known as coumarins, for example esculetin. Esculetin has been reported to have several pharmacological effects, including antioxidative activity.[21] Although several previous studies have evaluated the beneficial effects of chamomile in maintaining glycemic levels, the literature still lacks information regarding its action on salivary glands, especially its antioxidant effects.

The present study hypothesized that chamomile tea reduces hyperglycemia and prevents oxidative stress in the salivary glands of STZ-induced diabetic rats. Therefore, our study aimed to analyze the level of oxidative damage measured by MDA in the salivary glands while also evaluating the association between oxidative stress markers and antioxidant system activity in the salivary glands of diabetic rats with *ad libitum* consumption of chamomile tea.

## Methodology

The Animal Use Ethics Committee of the Faculty of Dentistry, University of São Paulo, approved the protocol for this study (process 015/2016). All experimental procedures were conducted in accordance with the instructions of the Brazilian College of Animal Care. Healthy male Wistar rats (200–270 g) were housed in individual cages, given access to rat chow and water or tea *ad libitum*, and maintained at a controlled temperature (23°C) with a 12/12 h light/dark cycle.

### Experimental protocol

The animals were randomly divided into four groups of seven rats each as follows: C, untreated control group that received only vehicle (water); CC, control group treated with chamomile tea instead of water; DM, untreated diabetic group receiving vehicle; and DMC, diabetic group treated with chamomile tea instead of water.

### Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of STZ (Sigma Chemical Co., St. Louis, USA) (60 mg/kg of body weight), always performed by the same operator. STZ was dissolved in a citrate buffer (pH 4.5). Control rats received the citrate buffer only. After 48 h, glycemia was determined in overnight-fasted animals by tail blood glucose analysis (Accu-Chek® Advantage, Roche Diagnostics, Basel, Switzerland). The rats in the diabetic group had blood glucose levels > 250 mg/100 mL. The animals were euthanized 28 days after the induction.

### Determination of chamomile tea composition by gas chromatography-mass spectrometry and ultraperformance liquid chromatography

*Matricaria chamomilla* (PMID: 18681440) dried flowers (100 g), obtained from a specialized tea store in bulk packaging, were hydrodistilled in a Clevenger-type apparatus for 3 h. The oil was collected by adding dichloromethane to a drop of oil in the device and then collecting the solution. Essential oil analysis was performed as described by Adams.[22] Individual compounds were identified by mass spectrometry (MS). Their identities were confirmed by comparing their retention indices and MS with authentic samples or data already available in the NIST 2005 Mass Spectral Library.

### Chamomile tea treatment

Chamomile tea was administered *ad libitum* as a substitute for water. Chamomile tea treatment was started one week after injection of STZ or citrate buffer and lasted 21 days. The chamomile tea was prepared daily by infusing the dried flowers in boiling tap water for 5 min, followed by filtration. After cooling, it was administered to the animals. A concentration of 200 mg/kg per day of chamomile tea was used. A previous study reported that this dose was effective in reducing hyperglycemia in STZ-induced diabetic rats.[17]

### Sample collection

Glucose levels were determined in overnight-fasted animals by tail blood glucose analysis (Accu-Chek® Advantage, Roche Diagnostics, Basel, Switzerland) on the last experimental day. The animals were anesthetized with an intraperitoneal injection of xylazine and ketamine (20/60 mg/kg body weight, respectively) in the morning (9:00–11:00). Blood was collected via heart puncture to analyze insulin levels. Blood and fat were immediately removed from the PA and SM glands, which were then frozen in liquid nitrogen and stored at −80°C until analysis.

### Insulin

Insulin concentration was evaluated via an immunoenzymatic assay using a commercial kit for rats/mice (Cat# EZRMI-13 K; Merck Millipore, Burlington, USA).

### Biochemical analyses

SOD, glutathione peroxidase enzymatic activities, and total antioxidant status in the glandular homogenate were determined using the commercial Ransod, Ransel, and total antioxidant status (TAS) test kits (Randox Laboratories, Crumlin, UK). CAT activity was assayed in accordance with the method described by Aebi.[23] Protein concentration was measured using the Folin phenol reagent. Bovine serum albumin was used as a standard.[24]

### Malondialdehyde (MDA)

MDA was assayed using HPLC. Chromatograms were monitored at 254 nm and the injection volume was 20 μL. The retention time of MDA was 3.14 min.[25] Protein concentration was measured using the Folin phenol reagent. Bovine serum albumin was used as a standard.[24]

### Statistical analysis

Data analysis was performed using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, USA). The Kolmogorov–Smirnov test determined the normality of the data. Results are presented as mean ± standard deviation (SD). The data were statistically compared via analysis of variance (ANOVA) followed by Tukey's test, where results were considered statistically significant at $p < 0.05$. Pearson's correlation was used to analyze the associations between the variables.

## Results

### Composition of chamomile tea

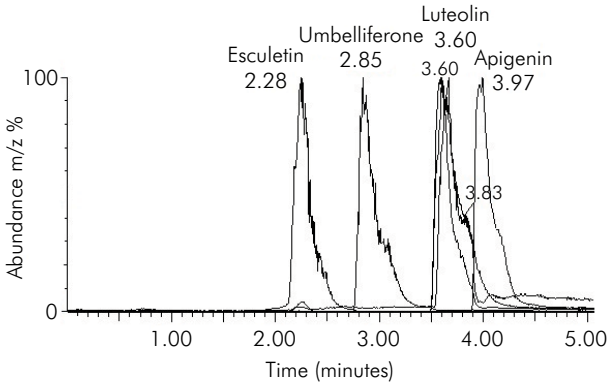
Gas chromatography-mass spectrometry (GC-MS) analysis confirmed the presence of 39 different constituents. The main constituents of the oil were alpha-bisabolol oxide A (21.70%), alpha-bisabolol oxide B (15.79%), 1,6-dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiynylidiene) (14.65%), β-farnesene (6.59%), alfa-bisabolol (5.58%), 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a. alpha.,4a.alpha,7.beta,7a.beta,7b.alpha) (4.48%), α-santalene (4.41%), benzene, 1,4-diethyl(2.71%),

malonic acid, isobutyl 2-methylpent-3-yl ester (2.13%), azulene, 7-ethyl-1,4-dimethyl-(2.13%) (Figure 1 ).

The phytochemical profile showed that *Matricaria chamomilla* tea is rich in several polyphenols and the major constituents were apigenin, luteolin, umbelliferone, and esculetin at the following concentrations: 33.23 ± 1.42, 2.98 ± 0.34, 2.64 ± 0.27 and 0.04 ± 0.02 µg/mL, respectively.

## Observations in rats

A significant increase in the initial glycemia was observed in both diabetic groups, whereas the glucose concentration remained the same in the control group throughout the study. The untreated diabetic group showed increased blood glucose concentration between the initial and final glycemic measurements. However, the high blood glucose levels decreased in the diabetic group treated with chamomile tea (Figure 2). Furthermore, a significant reduction in fasting blood insulin concentration was observed in the untreated diabetic and the treated diabetic groups compared to the untreated control ($p = 0.001$) and treated control groups ($p = 0.05$), respectively (Table. Additionally, the untreated diabetic group presented higher water consumption compared to the untreated control group. The same was observed in the treated diabetic group when compared to the control group, where the liquid consumption was, however, lower than that of the untreated diabetic group (Table).

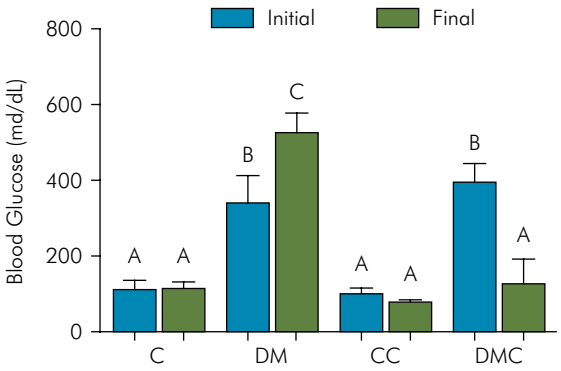



Rt: retention time sequence.

**Figure 1.** Gas chromatography-mass spectrometry (GC-MS) of *Matricaria chamomilla* extract. Targeted compounds are referred to according to the retention time sequence. The major constituents were found to be apigenin (3.97 min), luteolin (3.60 min), umbelliferone (2.85 min), and esculetin (2.28 min).

## Antioxidant profile and MDA quantification in the parotid gland

In the untreated diabetic group, the PA gland showed a reduction in the specific activity of SOD, GPx, CAT, and TAS compared to the control group. However, MDA levels were higher in the untreated group than in the control. Treatment with chamomile tea for 21 days resulted in a marked increase in the




C: control untreated group; CC: treated control group;
DM: untreated diabetic group; DMC: treated diabetic group.

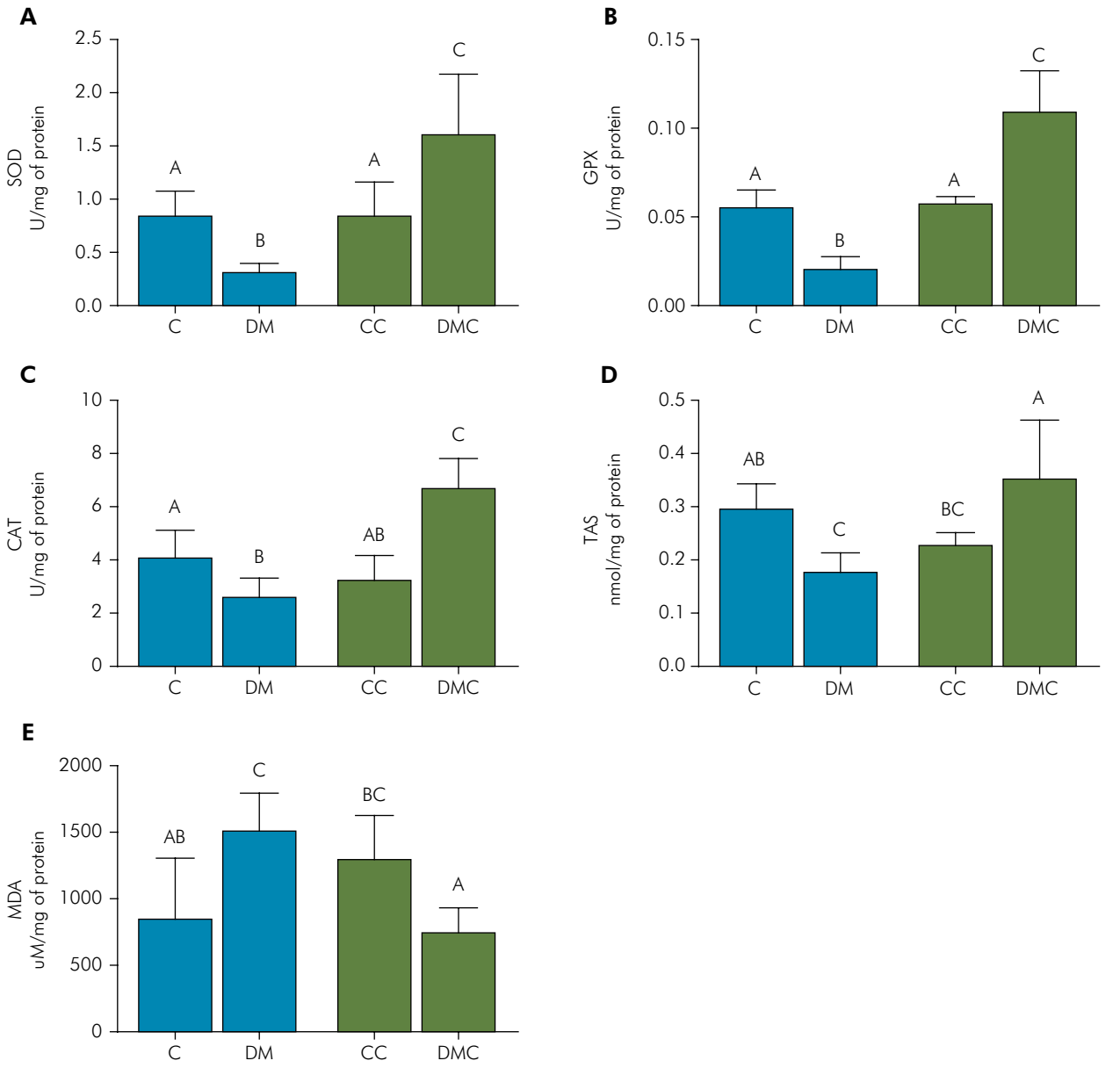
**Figure 2.** Initial and final blood glucose concentrations (mg/dL) in control and diabetic rats (30 days after streptozotocin induction) without and with supplementation of chamomile tea (200 mg/kg/day) for 21 days. Equal letters show similarities between the groups within each variable analyzed (one-way ANOVA with Tukey's test, $p < 0.05$). Each value represents the mean ± SD of experiments ($n = 7$).

**Table.** Insulin levels (ng/mL) and liquid consumption (water or tea) (mL/kg/day) in control and diabetic rats (30 days after streptozotocin induction) without and with supplementation of chamomile tea (200 mg/kg/day) for 21 days. Equal letters show similarities between the groups within each variable analyzed (one-way ANOVA with Tukey's test, $p < 0.05$). Each value represents the mean ± SD of experiments ($n = 7$).

| Variable | Insulin (ng/mL) | Liquid consumption (mL/kg/day) |
|---|---|---|
| C | 0.5338 ± 0.2173 (A) | 61.28 ± 15.75 (A) |
| DM | - | 145.6 ± 29.22 (B) |
| CC | 0.3100 ± 0.1837 (A) | 36.56 ± 15.20 (A) |
| DMC | - | 107.9 ± 28.69 (C) |

C: control untreated group; CC: treated control group;
DM: untreated diabetic group; DMC: treated diabetic group.

specific activities of SOD, GPx, CAT, and TAS in the diabetes-treated group compared to the control. In addition, it showed an increase in the specific activity of SOD, GPx, CAT, and TAS compared to the untreated diabetic group. Nevertheless, treatment with chamomile tea increased the activity of SOD, GPx, and CAT in the diabetic group compared to the control group. The levels of MDA in the PA glands of diabetic animals treated with chamomile tea significantly decreased compared to those in the untreated diabetic and treated control groups (Figure 3).

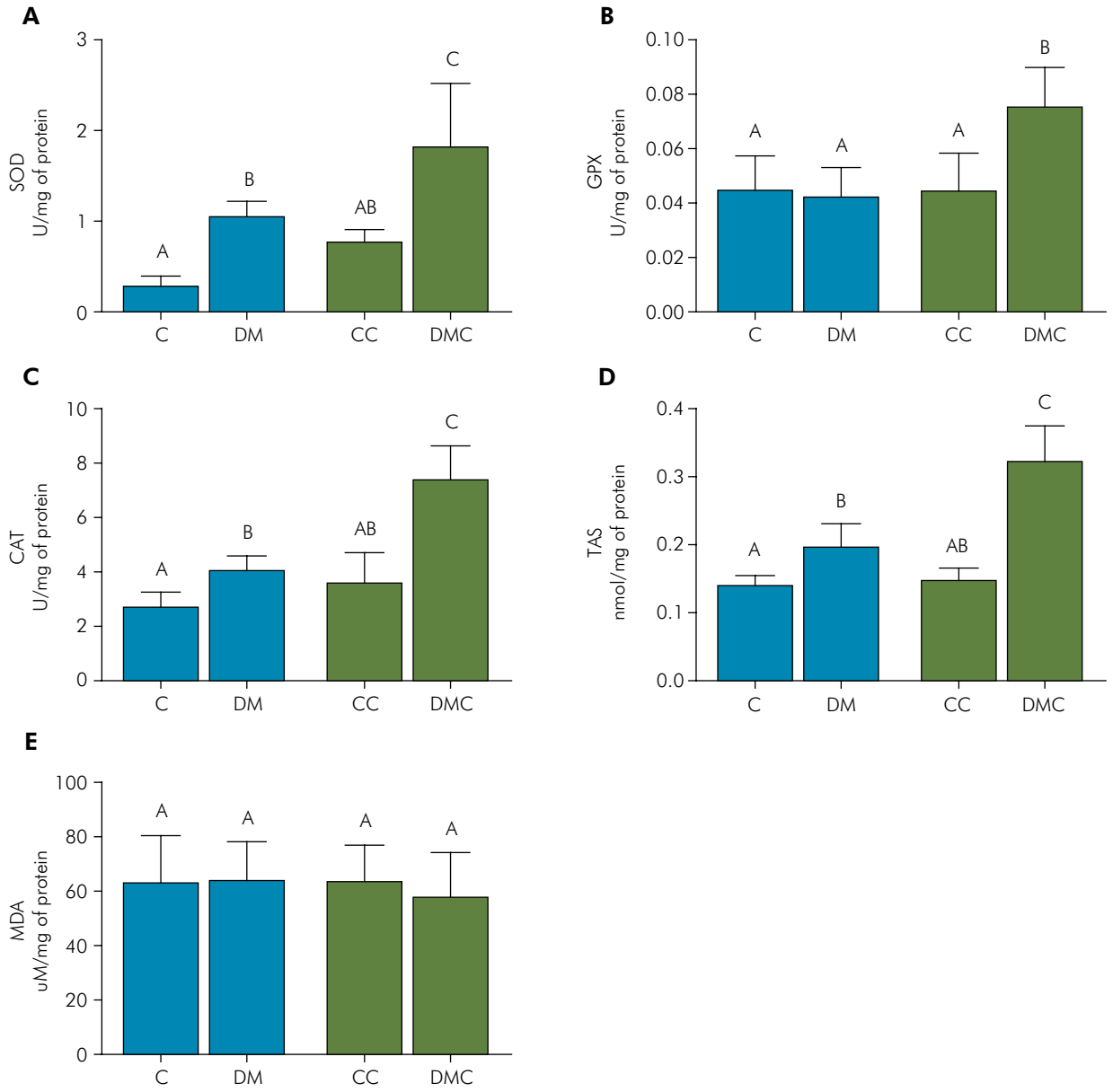



C: control untreated group; CC: treated control group; DM: untreated diabetic group; DMC: treated diabetic group.

**Figure 3.** (a) Superoxide dismutase (SOD), (b) glutathione peroxidase (GPx), (c) catalase activity, (d) total antioxidant capacity (TAS), and (e) Malondialdehyde (MDA) levels in parotid salivary glands (PA) of control and diabetic rats (30 days after streptozotocin induction) without and with supplementation of chamomile tea (200 mg/kg/day) for 21 days. Equal letters show similarities between the groups within each variable analyzed (one-way ANOVA with Tukey's test, $p < 0.05$). Each value represents the mean ± SD of experiments (n = 7).

### Antioxidant profile and MDA quantification in the submandibular gland

The SM showed an increase in the specific activity of SOD, CAT, and TAS in the untreated diabetic group compared to the control. Consumption of chamomile tea for 21 days resulted in a significant increase in the specific activities of SOD, GPx, CAT, and TAS in the diabetic-treated group compared to the control. In addition, the treated diabetic group showed increased specific activity of SOD, GPx, CAT, and TAS compared to the untreated diabetic and untreated control groups. No difference was observed in the MDA content (Figure 4).




C: control untreated group; CC: treated control group; DM: untreated diabetic group; DMC: treated diabetic group.

**Figure 4.** (a) Superoxide dismutase (SOD), (b) glutathione peroxidase (GPx), (c) catalase activity, (d) total antioxidant capacity (TAS), and (e) Malondialdehyde (MDA) levels in submandibular glands (SM) of control and diabetic rats (30 days after streptozotocin induction) without and with supplementation of chamomile tea (200 mg/kg/day) for 21 days. Equal letters show similarities between the groups within each variable analyzed (one-way ANOVA with Tukey's test, $p < 0.05$). Each value represents the mean ± SD of experiments ($n = 7$).

### Correlation

Pearson's correlation analysis showed a positive correlation between blood glucose concentration in the untreated diabetic group and the consumption of chamomile tea ($p = 0.0329$, $r = 0.63$). In the PA glands of the diabetic group, a positive correlation was also found between blood glucose concentration and MDA content ($p = 0.0413$, $r = 0.68$).

## Discussion

The main results confirm the hypoglycemic effect of the *ad libitum* consumption of chamomile tea. The data obtained showed a significant reduction in glycemia in the treated diabetic group compared to the untreated diabetic. In addition, we are first to demonstrate the antioxidant effect of chamomile tea treatment via the reduction of lipid peroxidation in the PA glands of diabetic rats and an increase in the enzymatic and non-enzymatic antioxidant system in both glands. These results suggest that *ad libitum* consumption of chamomile tea can prevent the progression of systemic hyperglycemia and glandular oxidative stress in the diabetic group.

DM is characterized by chronic hyperglycemia,[26] and perfect control of the blood glucose level is essential to prevent complications.[5] A previous study focused on new alternatives, such as chamomile, to prevent symptoms associated with diabetes.[16] In the diabetic animal model, several studies with chamomile showed many benefits, mainly attributed to its antioxidant and hypoglycemic effects when used at different concentrations and periods of treatment.[17,18,27] Cemek et al.[18] showed insulin-immunopositive cell density reduction in STZ-induced diabetic rats. Intensity of immunohistochemical staining of insulin Langerhans islets was preserved after therapy with *Matricaria chamomilla L.* ethanolic extract (MCE), which also decreased postprandial hyperglycemia after 14 days of treatment by gavage. In the present study, the administration of chamomile tea promoted a strong hypoglycemic effect, as evidenced by a significant reduction in glycemia in the treated diabetic group. Here, the consumption of chamomile tea also reduced the liquid consumption because of the osmotic rebalancing promoted by the hypoglycemic effect.

Although not statistically significant, the treated control group consumed ~40 % less tea than the control group. Treated control animals did not show any change in the parameters analyzed in the present study, suggesting that their hydroelectrolytic balance was unchanged. Further studies are necessary to evaluate the role of chamomile tea in this reduction. In our study, chamomile tea reduced blood glucose levels without changing the plasma insulin levels. This finding is pertinent and corroborates the results obtained in a previous study in which oral administration of ethanolic extract of chamomile for 4 weeks decreased serum glucose levels without significant improvement in serum insulin levels.[19]

In contrast, Najla et al.[17] showed an increase in blood insulin levels in diabetic rats treated with chamomile daily (200 mg/kg/day) for 3 weeks using a gastric cannula. The differences in these studies could be explained by the different experimental conditions, such as the STZ dose. Najla et al.[17] used a lower dose of STZ (45 mg/kg), and the factor for determining the extent of diabetogenic action is the dose of STZ.[17,18] Therefore, the possible mechanism by which chamomile tea significantly decreases glycemia levels is, in part, due to the inhibition of hepatic glycogen degradation.[27]

In the present study, we found a positive correlation between glycemia and MDA concentration in the PA glands of diabetic animals. Hyperglycemia has been widely reported to be responsible for a higher generation of free radicals and, when associated with reduced antioxidant defense capacity, leads to oxidative stress.[5,28,29] Oxidative stress is associated with reduced saliva secretion in STZ-induced diabetic rats,[12] leading to dry mouth symptoms, frequently reported in diabetic patients.[30] Furthermore, saliva is essential for maintaining oral health and integrity of oral cavity tissues.[31] However, one of the limitations of the present study is the absence of measurement of salivary flow and salivary analysis, making it impossible to evaluate the secretory function of the salivary glands and the effects of chamomile tea consumption.

Oxidative stress can be prevented by an enzymatic system responsible for eliminating excess ROS.[32] The enzymatic system is composed of SOD, responsible for catalyzing the dismutation of two molecules of superoxide anion in hydrogen peroxide and oxygen,[33] which is converted into water by CAT and GPx. Thus, both CAT and GPx prevent the accumulation of hydrogen peroxide.[34,35] TAS represents the sum of all antioxidants, and in the case of the salivary glands, it provides information on their antioxidant effectiveness.[36] These antioxidant enzymes play a significant role in preventing oxidative stress.[18]

It is important to emphasize that diabetes is related to decreased antioxidant capacity caused by reduced endogenous antioxidant enzyme activity and increased formation of ROS.[4] Our data showed a significant decrease in the total value of TAS, SOD, CAT, and GPx in the PA glands of untreated diabetic rats when compared to those of the untreated control group. The increased lipid peroxidation in the PA glands of untreated diabetic rats may be due to an inefficient antioxidant system.[13] The decrease in all enzyme activities, including TAS values, showed a reduced antioxidant potential, leading to oxidative stress, as confirmed by the increase in MDA content. The decrease in the defense mechanisms of enzymatic and non-enzymatic antioxidant systems may be due to increased use to remove free radicals. This evidence suggests that antioxidants are a promising additional treatment for DM.[15] Deconte et al.[11] showed an increase in the MDA content in the PA glands of diabetic rats 30 days after STZ induction. In addition, Zalewska et al.[13] demonstrated that the antioxidant defense of the PA glands is deficient throughout the duration of experimentally induced diabetes and suggested that changes in MDA depend on the type of salivary glands studied in rats.

The PA and SM salivary glands react differently in diabetic conditions.[10,12,38] Salivary glands present different metabolic pathways. In the PA glands, the main source of energy is aerobic metabolism, whereas in the SM, the main source is anaerobic glycolysis. According to the results obtained in this study and other studies 28 days after induction, the PA gland of untreated diabetic rats is subject to dysfunction in its antioxidant defense ability.[13,14]

A previous study showed the use of chamomile tea in patients with type 2 DM (3 g/150 mL hot water) three times per day immediately after meals for 8 weeks. The authors demonstrated a promising antioxidant agent that prevents oxidative stress by increasing antioxidant enzyme activities and reducing lipid peroxidation.[16] This study shows that chamomile tea promoted an improvement in the antioxidant capacity, defined by the increase in the enzymatic activity of SOD, GPx, CAT, and TAS in both glands and a marked reduction in lipid peroxidation in the PA glands of diabetic rats. Furthermore, the increased activity of antioxidant enzymes caused a reduction in lipid peroxidation in the treated PA diabetic gland when compared to the untreated. Thus, our results suggest that *ad libitum* consumption of chamomile tea has become the main tool for preventing oxidative stress in the PA glands of treated diabetic rats. In addition, chamomile tea contains high levels of polyphenolic compounds such as coumarins and flavonoids, which are related to the production of free radical scavengers.[27] Our findings were also compared with those of other studies that reported decreased serum MDA and increased CAT, GPx, and SOD activity and elevated TAS levels in diabetic rats after administration of chamomile ethanolic extracts[18,19] and chamomile tea.[16]

Although oxidative stress was observed only in the PA glands of untreated diabetic rats, in the SM glands, there was protection from damage caused by oxidative stress since there was no increase in MDA concentration. Therefore, this increase in the total antioxidant capacity in the SM glands may also be due to the increased activity of the enzymatic antioxidant system.

## Conclusions

The present study describes an important reduction in hyperglycemia and prevention of oxidative stress with 21 days of chamomile tea administration as a substitute for water, suggesting that chamomile tea can be a potential candidate for the prevention of oral complications in DM.

### Limitations of the study

In the present study, we evaluated the effect of chamomile tea on the antioxidant enzymes of the salivary glands and glycemia in STZ-induced diabetic rats. DM is characterized by high glucose concentrations and prolonged periods of hyperglycemia, leading to oxidative stress. However, we cannot postulate that the antioxidant effect is an indirect effect of chamomile tea on blood glucose reduction. Thus, further studies are required to confirm these findings.

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