https://doi.org/10.31925/farmacia.2024.6.23 **ORIGINAL ARTICLE**

PROBIOTIC EFFECTS ON OXIDATIVE STRESS PATHWAYS IN DIABETES

ANCA-IOANA (AMZĂR) SCĂRLĂTESCU [1], SORINA NICOLETA VOICU [2]*, MARIA-TEODORA PIȚURU [3], MIRUNA-MARIA APETROAEI [1], BRUNO ȘTEFAN VELESCU [1], DENISA IOANA UDEANU [1,4], MARINA-IONELA (ILIE) NEDEA [1], ANDREEA LETIȚIA ARSENE [1,4]

[1]*Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, 6 Traian Vuia Street, 020956 Bucharest, Romania*
[2]*Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Bucharest, Splaiul Independenței 91–95, 050095 Bucharest, Romania*
[3]*Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine, 105 Splaiul Independentei, 050097, Bucharest, Romania*
[4]*"Marius Nasta" Institute of Pneumology, Bucharest, Romania*

**corresponding author: sorina.voicu@bio.unibuc.ro*

Manuscript received: October 2024

Abstract

Diabetes is a complex metabolic disorder characterized by chronic hyperglycaemia and associated with significant oxidative stress, which contributes to the progressive damage of vital tissues. This study evaluates the potential benefits of probiotics in managing oxidative stress by administering them to both untreated diabetic groups and diabetic groups receiving standard antidiabetic therapy. Oxidative stress markers were assessed in the brain to explore the gut-brain axis and its connection to microbiota enrichment, while liver analysis provided insights into peripheral impact. The results revealed significant strain-specific variations in key parameters, highlighting the potential of probiotics to regulate oxidative stress and complement antidiabetic therapy.

Rezumat

Diabetul este o tulburare metabolică complexă, caracterizată prin hiperglicemie cronică și asociată cu un stres oxidativ semnificativ, care contribuie la deteriorarea progresivă a țesuturilor vitale. Acest studiu evaluează beneficiile potențiale ale probioticelor în gestionarea stresului oxidativ prin administrarea acestora atât la grupuri diabetice netratate, cât și la grupuri diabetice tratate cu terapie antidiabetică standard. Markerii stresului oxidativ au fost analizați în creier pentru a explora axa intestin-creier și conexiunea acesteia cu îmbogățirea microbiotei, în timp ce analiza ficatului a oferit informații despre impactul periferic. Rezultatele au evidențiat variații semnificative, specifice tulpinilor de probiotice utilizate, subliniind potențialul acestora de a regla stresul oxidativ și de a completa terapia antidiabetică.

Keywords: probiotics, oxidative stress, diabetes, oxidative stress marker, GSH, AOPP, MDA

Introduction

Diabetes mellitus is a group of life-long metabolic conditions characterized by high blood glucose levels regardless of the patient's fasted or postprandial state, resulting either from reduced or absent insulin secretion or its impaired action [1]. Diabetes is a metabolic and inflammatory disease. As the gut microbiota is an important metabolic regulator and also plays a crucial role in the inflammatory state of the entire body, it is not surprising that a correlation between diabetes and the health status of gut microbiota exists. The dietary fibres, which are the main energy source of intestinal microorganisms, are inversely correlated with the incidence of type 2 diabetes mellitus (T2DM). Non-digestible carbohydrates positively influence diabetes and also prevent its onset by lowering the glycaemic index of food and bringing anti-inflammatory SCFAs to the organism through the fermentation process of gut microbiota [2]. SCFAs likely increase the GLP-1 intestinal synthesis, which improves satiety and regulates glucose tolerance and homeostasis. Also, these agents could cause an increase in leptin and peptide YY release, which positively impacts satiety [3]. Therefore, the intestinal microbiota determines numerous metabolic effects that support the maintenance of physiological levels of blood sugar, and the interest in its potential applications in prevention and treatment of T2DM is increasing.

Intestinal microbiota dysbiosis manifested *via* an increased gut permeability is supposed to be related to the onset of many metabolic disorders, including diabetes. Once the intestinal barrier is damaged, the bacterial lipopolysaccharides can easily enter the systemic circulation, producing low-grade inflammation associated with metabolic endotoxemia. There are commensal

bacteria like the *Bifidobacterium* species and the *Faecalibacterium prausnitzii* that are negatively associated with diabetes and also possess anti-inflammatory properties [4].

In recent years, probiotics have gained significant attention for their potential role in managing various health conditions, ranging from gut health and metabolic disorders to immune regulation and even mental health, reflecting their growing importance in both research and clinical practice [5-7]. A meta-analysis of the effects of probiotics in T2DM concluded that such a therapeutic intervention can improve the control of this metabolic disease by decreasing insulin resistance, glycosylated haemoglobin and basal blood glucose. Also, as gastrointestinal dysfunctions such as delayed digestion, constipation and diarrhoea are part of diabetes symptoms spectre and a sign of developing disease, a probiotic therapy approach to these symptoms may be a good strategy for fighting this disease [8].

An imbalance between the production of reactive oxygen species and antioxidants profile in a status known as oxidative stress that in turn leads to severe damage to the cell's components such as proteins, lipids and DNA [9]**.** Oxidative stress significantly contributes to the development of insulin resistance, as well as the onset and progression of T2DM [10]. According to a comprehensive review, the diabetes significantly decreases glutathione (GSH) levels, suggesting a compromised antioxidant defence system in diabetic individuals. Concurrently, diabetes leads to an elevation of two markers of oxidative stress: advanced oxidation protein products (AOPP) and malondialdehyde (MDA), a lipid peroxidation product. These findings indicate that diabetes not only impairs antioxidant capacities but also exacerbates oxidative damage within the body, potentially contributing to the progression of diabetes-related complications [11].

The heightened pathogenicity in diabetes may be rooted in a gradual degeneration of vital tissues driven by oxidative stress. In this context, this study aims to unveil the effect of different probiotic strains on a murine model of diabetes mellitus, by assessing the metabolic and oxidative stress markers in both medically treated and untreated condition. This dual approach aims to evaluate whether probiotics alone can regulate oxidative stress in untreated diabetes and/or enhance the effects of standard antidiabetic treatment. Also, this study explores the importance of the microbiota-gut-brain connection in the diabetes context *via* assessing important oxidative stress parameter levels in brain and liver samples. Through this experimental design, the present study seeks to provide deeper insights into the underlying mechanisms of the microbiota-gut-brain interaction, opening new approaches for microbiota-targeted therapies in the management of diabetes mellitus and hopefully alleviating healthcare professionals confusion when it comes to combining diabetic and probiotic treatments.

Materials and Methods

Animal Selection and Housing Conditions

A group of 170 Wistar adult rats was used as the experimental subjects. These animals were obtained from the bio-base at the "Cantacuzino" National Institute of Medical and Military Research and Development, Romania. Their body weight was 150 - 200 g on the arrival date. All animal procedures took place in a controlled laboratory setting with regular day-night cycles, where temperature and humidity were carefully monitored, keeping their levels between 20 - 22°C, and respectively within 35 - 55%. The rats received a standard pellet diet and water ad libitum until the beginning of the experiment. To avoid disturbances in rats' physiological status and also to ensure the stabilization of possibly altered microbiota following animal transportation, the rats were acclimated for one week before starting the experimental procedure [12, 13]. The experimental research was conducted following Directive 2010/63/EU, Romanian Law 43/2014 regarding the use of animals for scientific purposes and ARRIVE guidelines of the National Institutes of Health. The ethic approval of study procedures and protocol was received from the Ethical Committee for Scientific Research of the "Carol Davila" University of Medicine and Pharmacy Bucharest, Romania.

Tested Compounds and Dosages

The study firstly involved inducing diabetes in rats, a model of disease which was obtained *via* administration of 130 mg/kg i.p. (intraperitoneal) of alloxan, a widely known inducer of diabetes mellitus [14]. After diabetes onset on the animals, the experiment continued with administration of various compounds: probiotic bacteria (*Lactobacillus paracasei* CNCM I-1572), probiotic yeasts (*Saccharomyces boulardii* CNCM I-1079), combinations of probiotics (*Lactobacillus paracasei* CNCM I-1572 and *Saccharomyces boulardii* CNCM I-1079), a commercial probiotic drink (combination of *L. casei* Danone CNCM I-1518, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) and metformin. The doses of these compounds were administered as follows: 500 μL of bacterial suspension containing 10^7 CFU/day *per* os (p.o.) [30, 31], 1 mL probiotic drink and 100 mg/kg of metformin *per* os. The bacterial and metformin suspensions were freshly prepared each day of the experiment.

Experimental Design and Group Allocation

When the acclimatization period was finished, 10 animals were randomly allocated to the control group. The rest of the rats fasted for 48 hours before and 24 hours after they were administered with intraperitoneal injection with a freshly prepared solution of alloxan, to ensure the proper effect of the diabetes-inducing agent. After 48 hours from injection, the onset of diabetes was tested in all animals, by measuring the fasting blood glucose (FBG) using a glucometer. The rats having

FBG over 200 mg/dL were considered diabetic and were randomly included in the study groups.

After the induction of diabetes, the diabetic animals were randomly split into ten groups, each containing 10 rats. Rats from the same group were housed in two cages, with 5 rats *per* cage, to avoid a crowded environment and eventual stress. The animals received their treatment *per* their assigned experimental groups. Throughout the experiment, their blood sugar and body weight were consistently monitored as important indicators of rats' response to their developing disease and response to treatment.

The study lasted for 3 weeks (21 days). This period was chosen because of numerous studies showing that probiotics, when taken regularly over a few days to weeks, eventually affect the structure and diversity of the gut microbiota and modulate its function [6]. Groups 1 to 10 were diabetic rats. Two of the diabetic groups were used as reference, as follows: The D group (diabetes control) received only physiologic solution and the DM group (treated/medicated diabetes) received therapy with metformin. DSL, DS, DL and DP groups received probiotic suspension as follows: DSL group – combined therapy with *Lactobacillus paracasei* and *Saccharomyces boulardii* suspension, DS group – *Sacharomyces boulardii* suspension, DL group – *Lactobacillus paracasei* suspension, DP group – commercial probiotic drink containing mainly *L. casei*. DMSL, DMS, DML and DMP groups received metformin and probiotic suspension as follows: DMSL group – metformin and combined therapy with a *Lactobacillus paracasei* and *Saccharomyces boulardii* suspension, DMS group – metformin and *Sacharomyces boulardii* suspension, DML group – metformin and *Lactobacillus paracasei* suspension, DMP group – metformin and commercial probiotic drink containing mainly *L. casei*. The control group (healthy control/ absolute control) received a physiological saline solution. Throughout the experimental period, all treatments were administered once a day, at the same time every day. Animals were sacrificed after 24 hours following the last day of the experiment. The rats were euthanized through decapitation to collect tissue samples for further examination. Specifically, the brain and liver were collected.

Organ samples processing for analyses

For preparation of organs homogenates, the tissues were weighed out accurately (approximately 0.2 g), then finely chopped with a sterile scalpel on a clean surface to increase the surface area, which helps in the homogenization process. The chopped tissue was transferred into a tube and a lysis buffer (pH = 7,4) was added, using a ratio of 10 volumes of buffer to 1 volume of tissue. The mixture was then homogenized 3 times for 30 seconds at high speed, using an UP50H ultrasonic processer, ensuring that no visible chunks remained after this procedure. To prevent heat damage of proteins, the samples were processed in an ice bath. The sonicator tip was cleaned with a wipe and ethanol before and after every use. For accuracy, the homogenates were then centrifugated at 10000 rpm and 4°C for 20 minutes to remove unbroken cells and eventual debris, retaining the supernatant in small aliquots. The homogenates were stored at -80°C until further analysis.

Oxidative stress markers analysis

This study evaluated the effects of probiotics on oxidative stress markers in a murine model of diabetes mellitus, aiming to determine their potential in restoring gut microbiota balance and reducing oxidative stress commonly associated with diabetes. Oxidative stress markers were analysed in brain and liver tissues to explore the interplay between these two key sites of oxidative damage and the potential impact of probiotic supplementation.

The concentrations of GSH, AOPP and MDA in brain and liver homogenates were measured using standard methods previously described in the literature. The method for determining reduced GSH was based on the protocol outlined by Roberts and Francetic [15]. The measurements of AOPP were determined *via* the Witko-Sarsat method [16], while MDA levels were analysed *via* the thiobarbituric acid reaction as described by Ohkawa *et al*. [17]. Protein content was assayed by the Bradford method using bovine serum albumin (BSA) as standard [18]. These methods were chosen for their established validity and widespread application in similar studies. The results for oxidative stress markers were expressed in nmol/mg protein.

Statistical Analysis

A one-way analysis of variance (ANOVA) was conducted on the data obtained from this experimental model, followed by Tukey's and Dunnett's post hoc test for multiple comparisons. Statistical analyses were performed using the GraphPad software. Results are presented as mean ± standard deviation (SD), and differences between groups were deemed statistically significant when the p value was less than 0.05.

Results and Discussion

Our disease model proved effective for studying how probiotics regulate brain and liver oxidative stress in the context of diabetes. The antioxidant activity, measured by reduced GSH levels in brain and liver tissues, was significantly impaired in diabetic rats compared to healthy controls. This was accompanied by an increase in protein oxidation and lipid peroxidation, as evidenced by elevated AOPP and MDA levels in the aforementioned tissues of diabetic rats as opposed to normal controls. Analysis of brain tissues revealed statistically significant differences between the healthy control group and the diabetes group, with decreased GSH levels ($p < 0.0001$) and increased AOPP ($p < 0.001$) and MDA levels ($p < 0.0001$) in the diabetes group, as shown in Figure 1a, Figure 1b and Figure 1c. Additionally, the diabetes

model effectively demonstrated alterations in hepatic antioxidant activity and oxidative stress parameters. Significant differences between the healthy control and diabetes groups included reduced GSH levels ($p < 0.0001$) and elevated AOPP levels ($p < 0.0001$) in the diabetes group. MDA levels in the diabetes group also showed a small but statistically significant decrease ($p < 0.05$), as *per* Figure 1d, Figure 1e and Figure 1f.

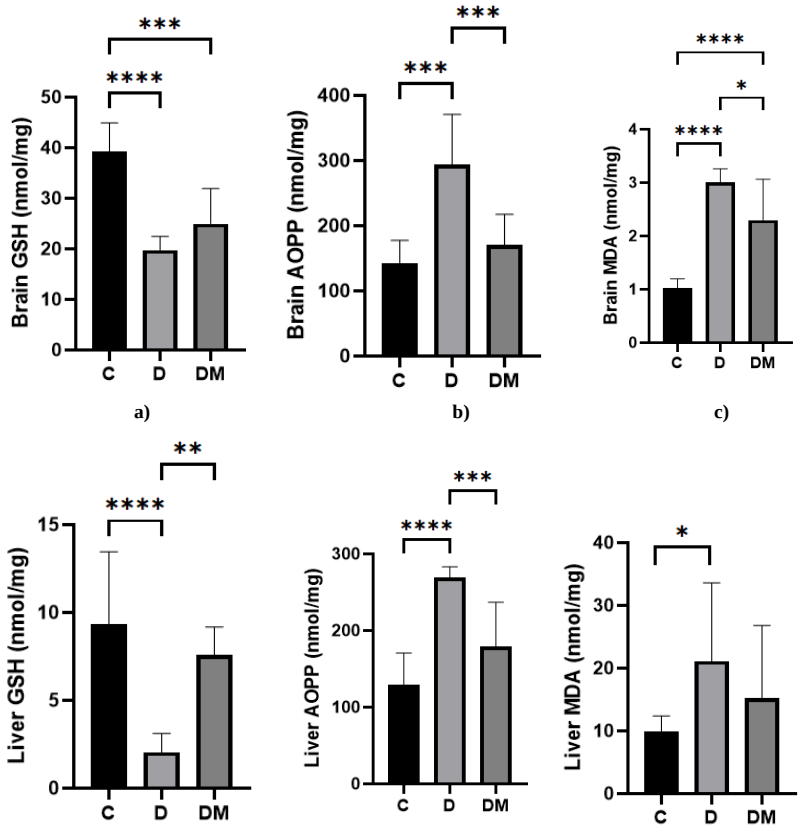

Figure 1.
a) Brain GSH variations; b) Brain AOPP variations; c) Brain MDA variations
d) Liver GSH variations; e) Liver AOPP variations; f) Liver MDA variations

Our results align with those of previous studies that reported decreased levels of reduced glutathione (GSH) and elevated concentrations of malondialdehyde (MDA) and AOPP in liver homogenates [19-23] of diabetic rodents. The same variations of stress parameters have been observed also in the brains of streptozocin or alloxan-induced diabetic rodents, confirming the cerebral oxidative toxicity caused by this metabolic disease [24-27].

Brain GSH

Reduced GSH levels disrupt mitochondrial function, compromising ATP production and thus restricting optimal cellular and physiological functions in diabetic conditions [28]. GSH in the brain comes from a combination of local synthesis within the astrocytes using amino acid precursors and BBB transport from the plasma [29]. Upon analysing GSH concentrations in the brain, considerable differences emerged between the diabetic control (D) and the other treatment groups. Metformin offers neuroprotective effects against oxidative stress induced by type 2 diabetes in the brain, as it has been reported to reduce stress markers like TBARS and MDA, decrease the activities of GPx and GRed enzymes and increase levels of reduced GSH [30]. This suggests that metformin, the gold-standard medication for type 2 diabetes, not only improves glucose regulation but also helps restore antioxidant defences in the brain, thereby mitigating oxidative damage. However, in our study, no significant improvement in brain GSH levels was observed in the Metformin-treated group (DM) compared to the untreated diabetic group (D), as illustrated in Figure 1a. Both groups showed a highly significant reduction in antioxidant activity compared to the Control group. This lack of improvement may be attributed to the short duration of the experiment, which may not have allowed sufficient time for Metformin to exert its effects on GSH levels. In contrast, the DMSL group showed a highly significant increase in GSH levels ($p < 0.0001$), with concentrations rising by approx. 65% compared to the D group, as presented in Figure 2a. Similarly, the DP group ($p < 0.001$) demonstrated a notable improvement, while the DML ($p < 0.01$) and DMP ($p < 0.05$) groups exhibited increases of up to 50 a respectively 40% relative to the D group, as shown in Figure 2a. These findings suggest that

Lactobacillus probiotics, whether used singly (*L paracasei*, DML group), in combination with probiotic yeast (*L paracasei* and *Saccharomyces boulardii*, DMSL group) or processed into specialized formulations (*L. casei*, DP and DMP groups), have a positive impact on brain GSH levels in both untreated and standard-treated diabetic models. This aligns with findings from another study, which reported that *L. plantarum* and *L. fermentum* can improve brain GSH levels following the administration of pro-inflammatory lipopolysaccharides [31]. This is a particularly important effect, as in diabetes, gut microbiota disturbances can weaken the gut barrier, allowing lipopolysaccharides (LPS) from Gram-negative bacteria to enter the bloodstream. If the blood-brain barrier (BBB) is also compromised, as often occurs in diabetes, LPS can reach the brain, triggering neuroinflammation, oxidative stress and neuronal damage [32-35]. Diabetes is closely associated with an increased risk of neurodegenerative diseases, due to oxidative stress and insulin resistance of the brain [36]. Notably, a study investigating the effects of *Lactobacillus plantarum* on aluminium-induced neurotoxicity reported significant findings. Administration of this probiotic led to substantial increases in antioxidant enzyme activities, including glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT). Additionally, glutathione (GSH) levels were elevated, while malondialdehyde (MDA), was significantly reduced [37]. Similarly, our study findings corroborated these results, showing a comparable increase in GSH levels and a reduction in MDA, highlighting the antioxidant effect of *Lactobacillus* strains and their combinations. Compared to the metformin-treated group (DM), a statistically significant difference ($p < 0.05$) was identified only in the DMSL group, although all lactobacilli-treated groups showed some degree of increased antioxidant activity, as shown in Figure 2b. These results indicate that the synergistic combination of probiotic bacteria and yeast may enhance cerebral GSH levels in animals undergoing conventional treatment. Conversely, *Saccharomyces boulardii*, as a standalone probiotic, demonstrates minimal beneficial effects on brain glutathione (GSH) levels in diabetic models included in our study.

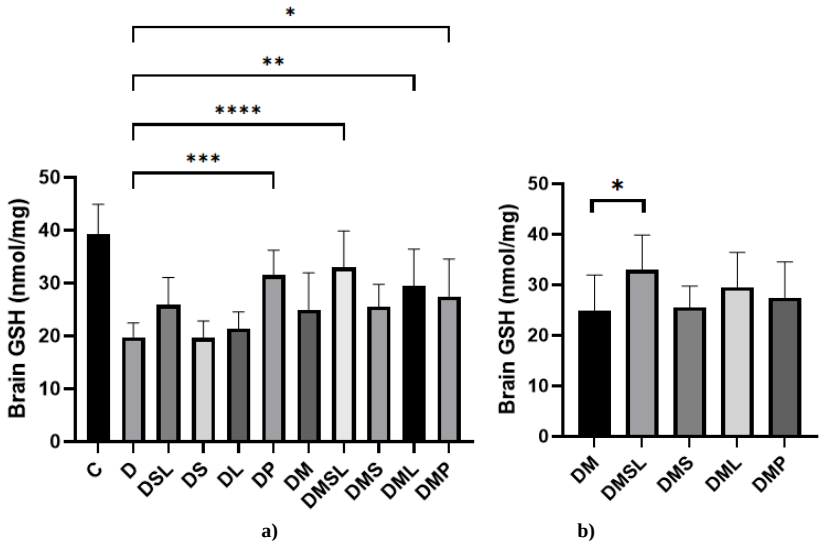

Figure 2.
Probiotics effect on brain GSH of a) untreated and treated diabetic animals relative to diabetic control; b) treated diabetic animals relative to metformin-treated diabetic control

Brain AOPP

Advanced oxidation protein products (AOPPs) form in diabetes due to the body's weakened antioxidant capacity, including reduced levels of protective –SH groups, which fail to counteract the heightened oxidative stress associated with high blood sugar and glycoxidation. This oxidative imbalance promotes protein damage and contributes to diabetes complications [38, 39]. Elevated levels of AOPPs in the brain tissue of animals with type 2 diabetes indicate significant oxidative stress, contributing to cognitive dysfunction associated with the disease [40]. Our study reveals important variations in AOPP levels across all probiotic groups. Compared to the D group, all groups receiving both antidiabetic and probiotic therapy showed highly statistically significant reductions in AOPP levels, as seen in Figure 3a, with general decreases ranging between 40% and 50%. Notably, all the probiotics combined with the antidiabetic drug (DMSL, DMS, DML, DMP groups) demonstrated a potent effect in reducing protein oxidation processes ($p < 0.0001$). Metformin monotherapy achieved AOPP concentrations nearly equivalent to those observed in the healthy control group, demonstrating that standard therapy is very effective in modulating brain oxidation in diabetic individuals. Indeed, metformin shows considerable

activity against elevated AOPP markers, in the brain of diabetic rats, underscoring its neuroprotective effects and potential application for diabetic patients at risk of complications such as haemorrhagic stroke [41]. Interestingly, even in the absence of antidiabetic treatment, all probiotic interventions significantly reduced AOPP concentrations compared to the diabetes model group. Specifically, the DL and DSL groups demonstrated significant reductions ($p < 0.001$), while the DS and DP groups also showed smaller yet notable decreases ($p < 0.01$), as per Figure 3a. However, when probiotics were administered alongside an antihyperglycemic drug, brain AOPP levels showed similar profiles across all groups, with no significant differences observed compared to the DM group, as *per* Figure 3b. These findings suggest that probiotic interventions may be beneficial for managing oxidative stress in undiagnosed or untreated diabetes, but do not significantly impact brain AOPP levels in diabetic animals already receiving standard antidiabetic therapy. This aligns with scientific literature indicating that AOPP levels exhibit an upward trend in chronic diabetes, even under glycaemic control, particularly during the first five years of the disease. After this period, AOPP levels tend to stabilize at a relatively constant level [42]. Therefore, probiotics may demonstrate greater efficacy when used as part of a longer-term therapeutic approach in conjunction with metformin, particularly during the early stages of diabetes when oxidative stress markers are more dynamic and responsive. Additionally, AOPPs are associated with ischemic and haemorrhagic stroke, contributing to oxidative stress and vascular damage in the brain [43]. Our study demonstrates the ability of probiotics to reduce AOPP levels, highlighting their therapeutic potential in lowering stroke risk and preserving brain health, particularly in conditions such as diabetes.

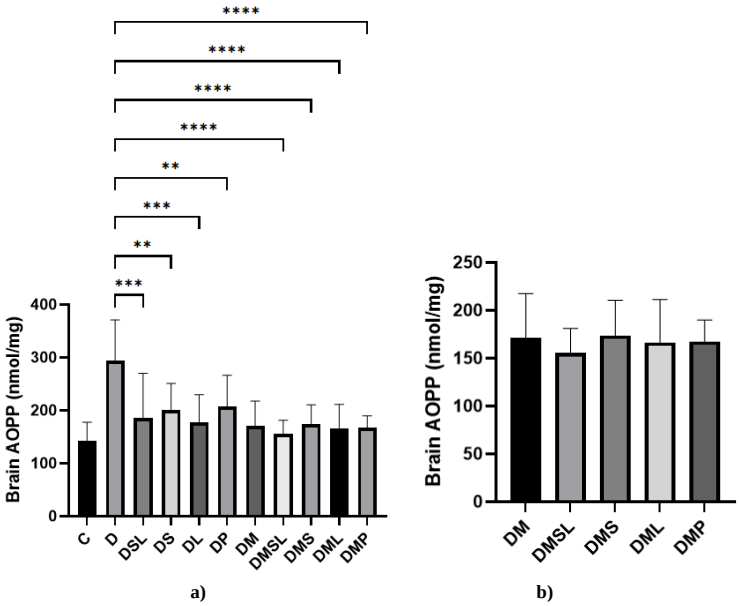

Figure 3.
Probiotics effect on brain AOPP of a) untreated and treated diabetic animals relative to diabetic control; b) treated diabetic animals relative to metformin-treated diabetic control

Brain MDA

Elevated lipid peroxidation in the diabetic brain is closely associated with reduced cellular energy and aging-related degeneration [28]. When comparing the D group to those receiving both antidiabetic treatment and probiotics, significant reductions in brain MDA levels were observed. The DMS, DML and DMA groups showed highly significant decreases ($p < 0.0001$), with reductions averaging 70% compared to the D group, as illustrated in Figure 4a. A smaller, yet significant reduction of approximately 40% was noted in the DMSL group ($p < 0.001$). In contrast, among diabetic groups without metformin therapy, only the *Saccharomyces*-treated group (DS) showed a marginal change ($p > 0.05$), with no notable differences observed in the other probiotic-treated groups. Using an animal model of neuroinflammation, another research showed that *Saccharomyces boulardii* determined a reduction of lipid peroxidation in two regions of the brain, the hippocampus and cortex [44]. Considering that neuro-inflammation and oxidative stress are implicated in diabetic encephalopathy [45], and our study showed that *Saccharomyces* reduces MDA levels in diabetes, these findings open the possibility that *Saccharomyces* may play a protective role in preventing neural damage in diabetic conditions. However, further studies are needed to explore this potential connection. In another study, treatment with *Lactobacillus plantarum* exopoly-saccharides in streptozotocin-induced diabetes significantly reduced hippocampal MDA levels, achieving results

comparable to those observed with the standard antidiabetic therapy, metformin [46]. In contrast, our study found that lactobacilli did not significantly reduce MDA concentrations unless administered alongside antidiabetic therapy. However, the DMS, DML and DMA groups demonstrated a significant decrease in MDA levels ($p < 0.0001$), with reductions of approx. 60% compared to the group receiving only standard metformin therapy, as shown in Figure 4b. Both the diabetes reference group and the metformin-treated diabetes group exhibited significantly elevated brain MDA levels relative to the healthy control. However, the reduction in MDA levels observed with Metformin alone was less substantial ($p < 0.05$) within the time-frame of this experiment, as *per* Figure 1c. These findings suggest that the observed decrease in oxidative stress, measured through cerebral MDA levels, is primarily attributable to the addition of probiotics to standard antidiabetic therapy. In diabetes, gut microbiota remains altered even with proper glycaemic control, characterized by reduced diversity, impaired SCFA production and a higher *Firmicutes*/*Bacteroidetes* ratio. Pro-inflammatory species like *Clostridia* and *Blautia* increase, while beneficial bacteria such as *Akkermansia*, *Bifidobacterium* and *Lactobacillus* decrease, further influenced by antidiabetic medications [47]. Given this, adding suitable probiotics for diabetic patients could enhance gut-brain axis activity and regulate brain oxidative stress probably by restoring microbiota balance and boosting anti-inflammatory SCFA production. Furthermore, *Lactobacillus casei*, the primary constituent of the probiotic drink administered to the DMP group, demonstrated antioxidant and anti-inflammatory properties in an Alzheimer's disease mouse model. It effectively reduced malondialdehyde (MDA) levels while enhancing superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in the brain. These benefits were achieved by regulating gut microbiota balance and elevating neurotransmitter levels [48].

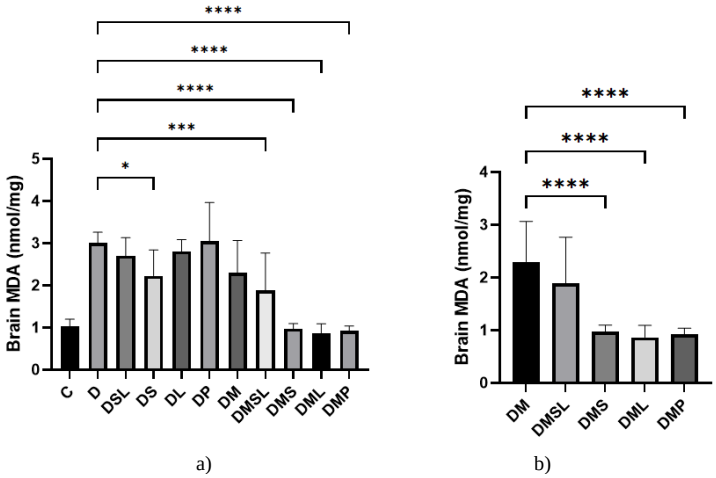

Figure 4.
Probiotics effect on brain MDA of a) untreated and treated diabetic animals relative to diabetic control; b) treated diabetic animals relative to metformin-treated diabetic control

Liver GSH

Glutathione (GSH) is an antioxidant agent primarily synthesized in the liver and has an important role in cellular redox reactions, as it acts like a free radical scavenger. Furfaro *et al*. have shown that GSH levels are reduced in diabetic liver, which may both result from and contribute to increased oxidative stress. This decrease of reduced glutathione could stem from impaired synthesis, increased degradation due to oxidation, or a combination of these processes [49]. In our study, analysis of GSH concentrations in liver tissue from untreated diabetic animals revealed a statistically significant increase ($p < 0.01$) in the group receiving only the *Saccharomyces* strain, with levels rising up to 3-fold compared to the diabetic control group (D), as presented in Figure 5a. *S. boulardii* demonstrates antioxidant activity through its ability to scavenge intracellular reactive oxygen species (ROS), a property that may be attributed to the presence of active compounds such as vanillin and vanillic acid in its fermentation broth [50]. This activity may help mitigate oxidative stress and prevent the depletion of reduced GSH. For the other probiotics not combined with antidiabetic therapy, the increases in GSH concentrations were too modest to reach statistical significance. In contrast, highly significant differences were observed between the D group and those receiving both probiotics and anti-diabetic therapy. Specifically, the DML group demonstrated a threefold increase in GSH levels compared to the D group ($p < 0.001$). Furthermore, the other anti-diabetic and probiotic-treated groups (DMSL, DMS and DMP) exhibited even more pronounced increases ($p < 0.0001$), with GSH levels rising up to fivefold relative to the D group. As shown in Figure 1d, the DM group restored hepatic GSH levels to near-control levels after antihyperglycemic treatment. Probiotic

interventions further accentuated this increase. However, these changes were not statistically significant when compared to the DM group, as illustrated in Figure 5b. For individuals not receiving adequate antidiabetic treatment, *Saccharomyces* appears to be the most effective adjuvant for enhancing antioxidant activity in diabetes. Supporting this, another study found that *Saccharomyces boulardii* can alleviate the hepatic complications of diabetes and especially liver injuries induced by this condition. Its benefits were demonstrated *via* the reduction of protein oxidative processes and enhancement of glutathione peroxidase activity, thus reducing oxidative stress [51]. The protective effects of *Saccharomyces boulardii* on hepatocyte structure and oxidative stress are further supported by the study of Cunha *et al*., attributing these benefits to the modulation of gut microbiota populations [52].

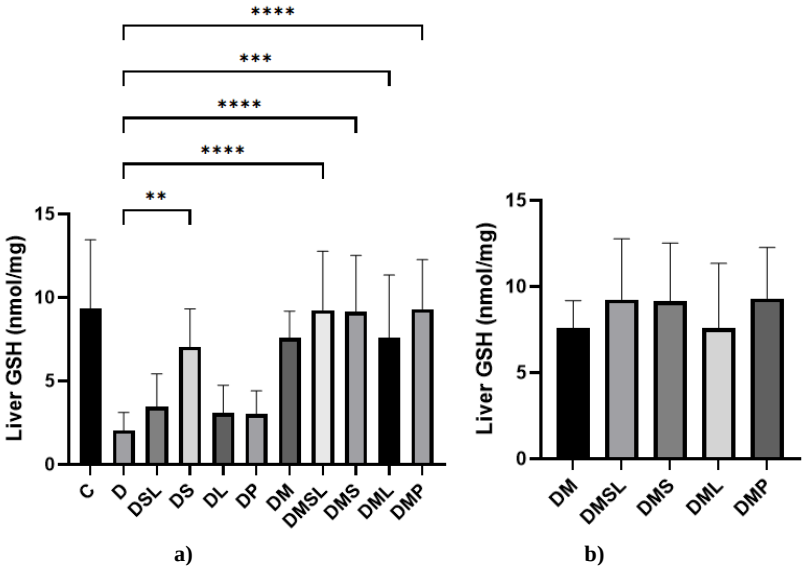

Figure 5.
Probiotics effect on liver GSH of a) untreated and treated diabetic animals relative to diabetic control; b) treated diabetic animals relative to metformin-treated diabetic control Liver AOPP

Advanced oxidation protein products (AOPPs) are markers of protein oxidative damage implicated in liver diseases. Their accumulation is heightened in diabetes, potentially worsening hepatic complications [53]. Regarding AOPP levels in diabetic groups, highly significant reductions averaging 40 - 50% compared to the D group, were found in the DSL, DMS, DML ($p < 0.0001$) and DMSL groups ($p < 0.001$), as *per* Figure 6a. Smaller, yet statistically significant decreases of up to 32% were detected in the DS and DP groups ($p < 0.01$) as well as the DL ($p < 0.05$) group. What is interesting is the probiotics' effects on alleviating protein oxidation at liver level in the absence of standard antidiabetic therapy, which is available for all the probiotics given, but especially for the group where the *Lactobacillus paracasei* and *Saccharomyces boulardii* were combined. *Lactobacillus plantarum* has been shown to reduce protein oxidation and lipid peroxidation in the liver, as well as decrease hepatic fat accumulation in non-alcoholic fatty liver disease [54], a condition highly prevalent among diabetes patients [55]. Notably, for anti-diabetes-medicated groups, only one statistically significant difference ($p < 0.01$) of 30% was observed between the AOPP levels of the DM group and the DML group, as shown in Figure 6b. As shown in Figure 1e, the group treated solely with metformin exhibited a highly significant reduction in liver protein oxidation compared to the diabetes reference group (D). However, the addition of the *Lactobacillus paracasei* strain further amplified this reduction, improving the modulation of oxidative stress even in the presence of standard therapy. Overall, all probiotic interventions led to a decrease in AOPP levels, except for the DMP group. Although the DMP group exhibited lower AOPP levels compared to the D group, its levels rose above those of the medicated diabetes reference group (DM). However, as there is no statistical difference, its use in the context of medicated diabetes is not necessarily questionable.

Liver MDA

MDA concentrations measured after the allocated treatments did not reveal statistically significant differences between the D group and the other groups, except for the group receiving both *Lactobacillus paracasei* and metformin (DML), as shown in Figure 7a. This group showed a significant reduction of approx. 65% ($p < 0.01$). Since metformin alone did not significantly reduce MDA levels in the DM group compared to the D group, it can be concluded that the addition of the *Lactobacillus* strain to standard antidiabetic therapy is a highly effective option for preventing hepatic lipid peroxidation. For diabetic groups

not receiving any antihyperglycemic therapy, none of the probiotic interventions – whether *Lactobacillus*, *Saccharomyces*, their combination, or a probiotic drink – demonstrated a statistically significant reduction in MDA concentrations, although all the probiotics decreased this parameter to some degree. No other significant reductions in MDA levels were observed between the DM group and its corresponding treatment groups, as *per* Figure 7b. Another study demonstrated the ability of the *Lactobacillus paracasei* to restore liver MDA levels in diabetic mice to those of healthy controls, a result that in our study happens only in the presence of metformin [56]. Similarly, a study investigating variations in hepatic and testicular oxidative parameters using the *Lactobacillus fermentum* and *Lactobacillus delbrueckii* combined with metformin for the treatment of streptozotocin-induced diabetes reported significantly reduced MDA levels and markedly increased GSH levels compared to the diabetic reference group [57]. Metformin has been found to increase *Lactobacillus* populations at the intestinal level [58]. Therefore, the combined use of antidiabetic drugs and probiotic bacteria may exert synergistic effects, enhancing glucose regulation, balancing gut microbiota and amplifying therapeutic benefits, including improved metabolic health and reduced oxidative stress in diabetes.

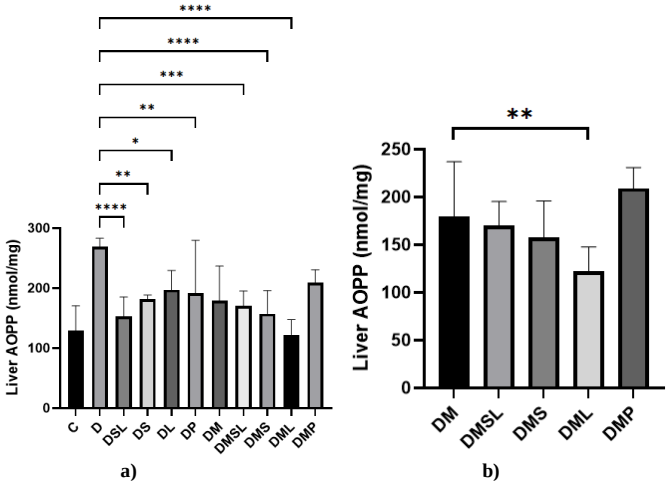

Figure 6.
Probiotics effect on liver AOPP of a) untreated and treated diabetic animals relative to diabetic control; b) treated diabetic animals relative to metformin-treated diabetic control

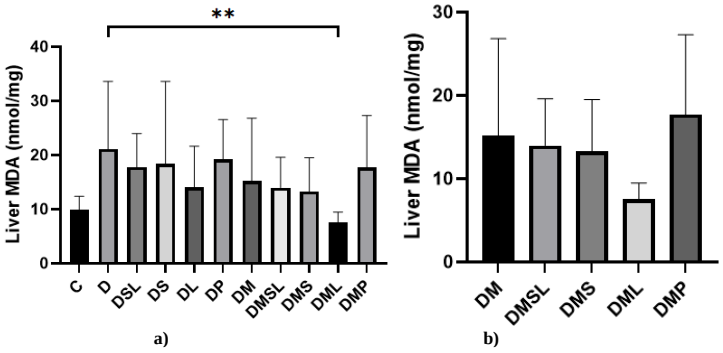

Figure 7.
Probiotics effect on liver MDA of a) untreated and treated diabetic animals relative to diabetic control; b) treated diabetic animals relative to metformin-treated diabetic control

The majority of the beneficial effects of probiotics on GSH levels in the liver and brain depend on their combination with antidiabetic therapy using metformin. In the brain, increased GSH levels are primarily attributed to probiotics containing *Lactobacillus* strains and their combination with *Saccharomyces boulardii*. Antioxidant activity is significantly enhanced when metformin is combined with the *Lactobacillus-Saccharomyces* duo (DMSL). In the liver, both *Saccharomyces* and *Lactobacillus*, either alone or combined, improve GSH levels, but only when administered alongside metformin. Interestingly, *Saccharomyces boulardii* demonstrates some independent antioxidant activity, increasing GSH levels on its own in the liver, while the probiotic drink exhibits a similar independent effect in the brain. However, the results show considerable variability between the two tissues analysed, highlighting the complex and tissue-specific nature of the probiotic effects.

The detrimental effects of oxidative stress on proteins were alleviated in both tested tissues, with a more pronounced reduction observed in the brain, following probiotic administration in both medicated and unmedicated diabetic groups. An exception was noted in the liver, where the probiotic drink failed to lower AOPP concentrations and instead caused a non-significant increase compared to the diabetic control group. However, in comparison to the metformin-treated reference group (DM), only the addition of *Lactobacillus paracasei* to the therapy demonstrated a positive effect in reducing protein oxidation processes. AOPPs are important markers of protein oxidative damage, whose accumulation contributes to development of atherosclerosis and the severe conditions such as aneurysms and the occlusion of critical arteries [59]. According to our study, probiotics significantly reduce AOPP levels in the brain and liver, thereby offering protection against the progression of atherosclerosis. This promising finding emphasizes the potential of probiotics to reduce the risk of atherosclerosis-related complications commonly seen in diabetes [60], safeguarding both the brain and liver.

Lipid peroxidation in the brain was significantly reduced in diabetic rats treated with metformin and all probiotic combinations (DMSL, DMS, DML, DMP) compared to untreated diabetic animals (D). However, the MDA-lowering effect of probiotics combined with the antidiabetic drug was not observed in hepatic tissue, except in the DML group. Furthermore, *Saccharomyces*, *Lactobacillus* and the probiotic drink, when added to standard antihyperglycemic treatment, led to a notable reduction in cerebral MDA levels. However, this effect was absent in liver tissue. For diabetic rats that did not receive any antidiabetic treatment, only *Saccharomyces* caused a significant reduction in brain lipid oxidation, while no reduction was observed in the liver across any probiotic treatment.

The results of this study provide evidence for the importance of the microbiota-gut-brain axis in managing oxidative stress. The gut microbiota profoundly influences brain function through this connection, modulating neural signalling, neurotransmitter production and inflammatory responses; however, dysbiosis, or an imbalance in gut microbiota, disrupts this communication, leading to increased neuroinflammation, altered neurotransmitter levels and cognitive impairments [61, 62]. Probiotics have been found to improve synaptic dysfunction and cognitive performance associated with the diabetic brain through mechanisms reliant on the microbiota-gut-brain axis [63]. Interestingly, in our study, some beneficial effects observed in the brain are not evident in the liver (*e.g.* MDA levels), suggesting that the microbiota has a more significant influence on the gut-brain axis compared to the gut-liver axis. This disparity may stem from the brain's heightened vulnerability to oxidative damage due to its high oxygen consumption, lipid content and relatively poor antioxidant enzyme load [64]. In diabetes, oxidative stress and inflammation in the brain are exacerbated by glucose autoxidation, lipid peroxidation and reduced antioxidant levels, such as glutathione [65]. The gut microbiota communicates with the brain through multiple pathways, including the vagus nerve and neuroactive signalling molecules such as short-chain fatty acids (SCFAs) which can cross the BBB and exert neuroprotective effects at the cerebral level [66]. Butyrate, one of the main SCFAs produced by microbiota inhibits oxidative stress and prevents ROS production increase by facilitating the release of NO through a mechanism dependent on the downregulation of G protein-coupled receptors (GPRs). This antioxidative effect is enhanced by dietary supplementation with probiotics, which promote butyrate production [67]. Also, the gut microbiota plays a significant role in brain chemistry and overall brain health by producing or directly contributing to the synthesis of a wide range of neurotransmitters [68]. These mechanisms could explain why the brain is particularly responsive to the beneficial effects of probiotics, compared to the liver. However, probiotics may alleviate oxidative stress in the liver through various mechanisms. For instance, an interesting mechanism proposed by Yan *et al*. for *Lactobacillus acidophilus*, a representative of the lactobacilli species, involves regulating the gut microbiota to enhance SCFA production. These SCFAs are transported to the liver *via* the portal vein, where they lessen inflammatory responses and regulate oxidative and metabolic stress [69]. A similar mechanism may explain the findings in our study, where probiotics appeared to influence oxidative stress markers in the liver, potentially through the modulation of gut microbiota and SCFA-mediated pathways.

Conclusions

Probiotics demonstrate a positive influence on oxidative stress in diabetic animals, notably reducing AOPP levels in the brain and liver, regardless of antidiabetic treatment. The most significant improvements in GSH and MDA levels were observed in animals receiving probiotics in conjunction with antidiabetic therapy, with GSH increasing in both the brain and liver and MDA reductions being particularly evident in the brain. However, probiotics showed beneficial effects even in untreated or undiagnosed diabetic conditions, reducing oxidative stress in both analysed tissues, as evidenced in this study. While these results are promising, further research is essential to clarify the underlying mechanisms, particularly the modulation of intestinal microbiota, and to validate the efficacy of specific probiotics in clinical trials. Overall, this study suggests that probiotic supplementation could offer a valuable strategy for reducing oxidative stress and protecting liver and brain health in diabetes and related conditions.

Conflict of interest

The authors declare no conflict of interest.

References

1. Alam U, Asghar O, Azmi S, Malik RA, General aspects of diabetes mellitus. *Handb Clin Neurol.*, 2014; 126: 211-22.
2. Spînu S, Orțan A, Voaideș C, Short review on bioactive compounds recovered from vegetal waste as a new tool in diabetes, obesity and dyslipidaemia prevention and treatment. *Farmacia*, 2022; 70(6): 1028-1036.
3. Bielka W, Przezak A, Pawlik A, The role of the gut microbiota in the pathogenesis of diabetes. *Int J Mol Sci.*, 2022; 23: 480.
4. Everard A, Cani PD, Diabetes, obesity and gut microbiota. *Best Pract Res Clin Gastroenterol.*, 2013; 27: 73-83.
5. Apetroaei MM, Fragkiadaki P, Velescu Bs, Baliou S, Renieri E, Dinu-Pirvu CE, Drăgănescu D, Vlăsceanu AM, Nedea MI, Udeanu DI, Docea AO, Tsatsakis A, Arsene AL, Pharmacotherapeutic considerations on telomere biology: the positive effect of pharmacologically active substances on telomere length. *Int J Mol Sci.*, 2024; 25: 7694.
6. Voicu SN, Scărlătescu AI, Amzăr, Apetroaei MM, Nedea MI, Blejan IE, Udeanu DI, Velescu B, Ghica M, Nedea OA, Cobelschi CP, Arsene AL, Evaluation of neuro-hormonal dynamics after the administration of probiotic microbial strains in a murine model of hyperthyroidism. *Nutrients*, 2024; 16: 1077.
7. Velescu B, Ștefan, Ilie MI, Amzăr AI, Lupașcu RE, Marandiuc IM, Apetroaei MM, Arsene AL, Blejan EI, Nedea OA, Fistos T, Fierăscu RC, Bărbuceanu F, Țoca C, Fierăscu I, Udeanu DI, Ghica M, Drăgănescu D, Cobelschi, Development and experimental evaluation of some silver nanoparticles with antimicrobial potential. *Processes*, 2023; 11: 1212.
8. Tao YW, Gu YL, Mao XQ, Zhang L, Pei YF, Effects of probiotics on type II diabetes mellitus: a meta-analysis. *J Transl Med.*, 2020; 18: 30.
9. Juan CA, Pérez de la Lastra JM, Plou FJ, Pérez-Lebeña E, The chemistry of reactive oxygen species revisited: outlining their role in biological macromolecules and induced pathologies. *Int J Mol Sci.*, 2021; 22: 4642.
10. Houstis N, Rosen ED, Lander ES, Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature*, 2006; 440: 944-948.
11. Yang H, Jin X, Kei Lam CW, Yan SK. Oxidative stress and diabetes mellitus. *Clin Chem Lab Med.*, 2011; 49.
12. Obernier JA, Baldwin RL, Establishing an appropriate period of acclimatization following transportation of laboratory animals. *ILAR J.*, 2006; 47: 364-369.
13. Montonye DR, Ericsson AC, Busi SB, Lutz C, Wardwell K, Acclimation and institutionalization of the mouse microbiota following transportation. *Front Microbiol.*, 2018; 9: 1085.
14. Lenzen S, The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, 2008; 51: 216-226.
15. Roberts JC, Francetic DJ, The importance of sample preparation and storage in glutathione analysis. *Anal Biochem.*, 1993; 211: 183-187.
16. Witko-Sarsat V, Friedlander M, Nguyen Khoa T, Capeillère-Blandin C, Nguyen AT, Canteloup S, Dayer JM, Jungers P, Drüeke T, Descamps-Latscha B, Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. *J Immunol.*, 1998; 161: 2524-2532.
17. Ohkawa H, Ohishi N, Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.*, 1979; 95: 351-358.
18. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.*, 1976; 72: 248-254.
19. Oyenihi OR, Brooks NL, Oguntibeju OO, Effects of kolaviron on hepatic oxidative stress in streptozotocin induced diabetes. *BMC Complement Altern Med.*, 2015; 15: 236.
20. Thomson M, Al-Qattan K, Mansour MH, Ali M, Green tea attenuates oxidative stress and downregulates the expression of angiotensin II AT1 receptor in renal and hepatic tissues of streptozotocin-induced diabetic rats. *Evid Based Complement Altern Med.*, 2012; 2012: 1-10.
21. Abdelrazek HMA, Kilany OE, Muhammad MAA, Tag HM, Abdelazim AM. Black seed thymoquinone improved insulin secretion, hepatic glycogen storage, and oxidative stress in streptozotocin-induced diabetic male Wistar rats. Oxid Med Cell Longev. 2018; 2018.
22. El-Missiry MA, El Gindy AM, Amelioration of alloxan-induced diabetes mellitus and oxidative stress in rats by oil of Eruca sativa seeds. *Ann Nutr Metab.*, 2000; 44: 97-100.
23. Cumaoğlu A, Cevik Ç, Rackova L, Ari N, Karasu Ç. Effects of antioxidant stobadine on protein carbonylation, advanced oxidation protein products and reductive capacity of liver in streptozotocin-diabetic rats: role of oxidative/nitrosative stress. *Biofactors*, 2007; 30: 171-178.
24. Sözbir E, Nazıroğlu M, Diabetes enhances oxidative stress-induced TRPM2 channel activity and its control by N-acetylcysteine in rat dorsal root ganglion and brain. *Metab Brain Dis.*, 2016; 31: 385-393.
25. Hussein J, El-Naggar ME, Fouda MMG, Othman SI, Allam AA, Nadwa EH, Rashwan EK, Hendawy OM, Eco-friendly microwave synthesis of gold nanoparticles for attenuation of brain dysfunction in diabetic rats. *J Clust Sci.*, 2021; 32: 423-435.
26. Hussein J, El-Matty DA, El-Khayat Z, Abdel-Latif Y, Therapeutic role of coenzyme Q10 in brain injury during experimental diabetes. *J Appl Pharm Sci.*, 2013; 3: 213-217.
27. Eltahawy NA, Saada HN, Hammad AS, Gamma amino butyric acid attenuates brain oxidative damage associated with insulin alteration in streptozotocin-treated rats. *Indian J Clin Biochem.*, 2017; 32: 207-213.
28. Mukherjee B, Mukherjee JR, Chatterjee M, Lipid peroxidation, glutathione levels and changes in glutathione-related enzyme activities in streptozotocin-induced diabetic rats. *Immunol Cell Biol.*, 1994; 72: 109-114.
29. Dwivedi D, Megha K, Mishra R, Mandal PK, Glutathione in brain: overview of its conformations, functions, biochemical characteristics, quantitation

and potential therapeutic role in brain disorders. *Neurochem Res.*, 2020; 45: 1461-1480.
30. Correia S, Carvalho C, Santos MS, Proença T, Nunes E, Duarte AI, Monteiro P, Seiça R, Oliveira CR, Moreira PI, Metformin protects the brain against the oxidative imbalance promoted by type 2 diabetes. *Med Chem.*, 2008; 4: 358-364.
31. Musa NH, Mani V, Lim SM, Vidyadaran S, Abdul Majeed AB, Ramasamy K, Lactobacilli-fermented cow's milk attenuated lipopolysaccharide-induced neuroinflammation and memory impairment *in vitro* and *in vivo*. *J Dairy Res.*, 2017; 84: 488-495.
32. Jayashree B, Bibin YS, Prabhu D, Shanthirani CS, Gokulakrishnan K, Lakshmi BS, Mohan V, Increased circulatory levels of lipopolysaccharide and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. *Mol Cell Biochem.*, 2014; 388: 203-210.
33. Lu J, Ma KL, Ruan XZ, Dysbiosis of gut microbiota contributes to the development of diabetes mellitus. *Infect Microbes Dis.*, 2019; 1: 43-48.
34. Banks WA, Gray AM, Erickson MA, Salameh TS, Damodarasamy M, Sheibani N, Meabon JS, Wing EE, Morofuji Y, Cook DG, Lipopolysaccharide-induced blood-brain barrier disruption: roles of cyclooxygenase, oxidative stress, neuroinflammation, and elements of the neurovascular unit. *J Neuroinflammation.*, 2015; 12: 223.
35. Brănescu C, Serban D, Dascălu AM, Oprescu SM, Savlovschi C, Interleukin 6 and lipopolysaccharide binding protein - markers of inflammation in acute appendicitis. *Chirurgia (Bucur)*, 2013; 108: 206-214.
36. Rosales-Corral S, Tan DX, Manchester L, Reiter RJ, Diabetes and Alzheimer disease, two overlapping pathologies with the same background: oxidative stress. *Oxid Med Cell Longev.*, 2015; 2015: 1-14.
37. Tian F, Yu L, Zhai Q, Xiao Y, Shi Y, Jiang J, Liu X, Zhao J, Zhang H, Chen W, The therapeutic protection of a living and dead Lactobacillus strain against aluminum-induced brain and liver injuries in C57BL/6 mice. *PLoS One*, 2017; 12: e0175398.
38. Pandey KB, Mishra N, Rizvi SI, Protein oxidation biomarkers in plasma of type 2 diabetic patients. *Clin Biochem.*, 2010; 43: 508-511.
39. Conti G, Caccamo D, Siligato R, Gembillo G, Satta E, Pazzano D, Carucci N, Carella A, Campo GD, Salvo A, Santoro D, Association of higher advanced oxidation protein products levels in patients with diabetic and hypertensive nephropathy. *Medicina (B Aires)*, 2019; 55: 675.
40. Aksenov MY, Aksenova MV, Butterfield DA, Geddes JW, Markesbery WR, Protein oxidation in the brain in Alzheimer's disease. *Neuroscience*, 2001; 103: 373-383.
41. Holubiev VL, Oberemok MH, Tkachenko VA, Kharchenko YV, Bondarenko OO, Lievykh AE, Study of the neuroprotective properties of metformin in rats with type 2 diabetes mellitus and brain injury induced by intracerebral hemorrhage. *Medicni Perspekt.*, 2024; 29(2): 174-186.
42. Heidari F, Rabizadeh S, Rajab A, Heidari F, Mouodi M, Mirmiranpour H, Esteghamati A, Nakhjavani M, Advanced glycation end-products and advanced oxidation protein products levels are correlates of duration of type 2 diabetes. *Life Sci.*, 2020; 260: 118422.
43. Toloue Pouya V, Hashemy SI, Shoeibi A, Nosrati Tirkani A, Tavallaie S, Zahedi Avval F, Serum pro-oxidant-antioxidant balance, advanced oxidized protein products and protein carbonyl in patients with stroke. *Razavi Int J Med.*, 2016; 4(2): e38203.
44. Babaei F, Navidi-Moghaddam A, Naderi A, Ghafghazi S, Mirzababaei M, Dargahi L, Mohammadi G, Nassiri-Asl M, The preventive effects of *Saccharomyces boulardii* against oxidative stress induced by lipopoly-saccharide in rat brain. *Heliyon*, 2024; 10: e30426.
45. Zeinivand M, Nahavandi A, Zare M, Deferoxamine regulates neuroinflammation and oxidative stress in rats with diabetes-induced cognitive dysfunction. *Inflammopharmacology*, 2020; 28: 575-583.
46. Qi Y, Wang D, Fang L, Liu X, Liu C, Zhao F, Wu D, Wang X, Wang J, Min W, Hypoglycemic effect of exopolysaccharide from *Lactiplantibacillus plantarum* JLAU103 on streptozotocin and high-fat diet-induced type 2 diabetic mice. *Foods*, 2022; 11: 3571.
47. Sircana A, Framarin L, Leone N, Berrutti M, Castellino F, Parente R, De Michieli F, Paschetta E, Musso G, Altered gut microbiota in type 2 diabetes: just a coincidence? *Curr Diab Rep.*, 2018; 18(10): 98.
48. Qiao L, Chen Y, Song X, Dou X, Xu C, Selenium nanoparticles-enriched Lactobacillus casei ATCC 393 prevents cognitive dysfunction in mice through modulating microbiota-gut-brain axis. *Int J Nanomedicine.*, 2022; 17: 4807-4827.
49. Furfaro AL, Nitti M, Marengo B, Domenicotti C, Cottalasso D, Marinari UM, Pronzato MA, Traverso N, Impaired synthesis contributes to diabetes-induced decrease in liver glutathione. *Int J Mol Med.*, 2012; 29(5): 899-905.
50. Suryavanshi A, Agarwal A, Kaler A, Bihade U, Kaur J, Tikoo K, Comparative studies on the antioxidant potential of vanillin-producing *Saccharomyces boulardii* extracts. *Oxid Antioxid Med Sci.*, 2013; 2: 201.
51. Barssotti L, Abreu ICME, Brandão ABP, Albuquerque RCMF, Ferreira FG, Salgado MAC, Dias DDS, De Angelis K, Yokota R, Casarini DE, Souza LB, Taddei CR, Cunha TS, *Saccharomyces boulardii* modulates oxidative stress and renin angiotensin system attenuating diabetes-induced liver injury in mice. *Sci Rep.*, 2021; 11: 9189.
52. Cunha TS, dos Santos LB, Abreu IME, Albuquerque RCMF, Brandão ABP, Ferreira FG, Hypoglycemic effect and hepatoprotective role of *Saccharomyces boulardii* THT 500101 strain in a murine model of streptozotocin-induced diabetes. *FASEB J.*, 2020; 34: 1-10.
53. Zhao Y, Zhang L, Ouyang X, Jiang Z, Xie Z, Fan L, Zhu D, Li L, Advanced oxidation protein products play critical roles in liver diseases. *Eur J Clin Invest.*, 2019; 49(6): e13098.
54. Li C, Nie SP, Zhu KX, Ding Q, Li C, Xiong T, Xie MY, *Lactobacillus plantarum* NCU116 improves liver function, oxidative stress and lipid metabolism in rats with high fat diet induced non-alcoholic fatty liver disease. *Food Funct.*, 2014; 5(12): 3216-3223.
55. Bhatt HB, Smith RJ, Fatty liver disease in diabetes mellitus. *Hepatobiliary Surg Nutr.*, 2015; 4: 101-108.

56. Zeng Z, Yuan Q, Yu R, Zhang J, Ma H, Chen S, Ameliorative effects of probiotic *Lactobacillus paracasei* NL41 on insulin sensitivity, oxidative stress, and beta-cell function in a type 2 diabetes mellitus rat model. *Mol Nutr Food Res.*, 2019; 63(22): e1900457.
57. El-Baz AM, Shata A, Hassan HM, El-Sokkary MMA, Khodir AE, The therapeutic role of *Lactobacillus* and montelukast in combination with metformin in diabetes mellitus complications through modulation of gut microbiota and suppression of oxidative stress. *Int Immunopharmacol.*, 2021; 96: 107757.
58. Zhou Z, Ren L, Zhan P, Yang H, Chai D, Yu Z, Metformin exerts glucose-lowering action in high-fat fed mice *via* attenuating endotoxemia and enhancing insulin signaling. *Acta Pharmacol Sin.*, 2016; 37: 1063-1075.
59. Gryszczyńska B, Formanowicz D, Budzyń M, Wanic-Kossowska M, Pawliczak E, Formanowicz P, Majewski W, Strzyżewski KW, Kasprzak MP, Iskra M, Advanced oxidation protein products and carbonylated proteins as biomarkers of oxidative stress in selected atherosclerosis-mediated diseases. *Biomed Res Int.*, 2017; 2017: 1-9.
60. La Sala L, Prattichizzo F, Ceriello A, The link between diabetes and atherosclerosis. *Eur J Prev Cardiol.*, 2019; 26: 15-24.
61. Strandwitz P, Neurotransmitter modulation by the gut microbiota. *Brain Res.*, 2018; 1693: 128-133.
62. Hosseinifard ES, Morshedi M, Bavafa-Valenlia K, Saghafi-Asl M, The novel insight into anti-inflammatory and anxiolytic effects of psychobiotics in diabetic rats: possible link between gut microbiota and brain regions. *Eur J Nutr.*, 2019; 58: 3361-3375.
63. Davari S, Talaei SA, Alaei H, Salami M, Probiotics treatment improves diabetes-induced impairment of synaptic activity and cognitive function: behavioral and electrophysiological proofs for microbiome-gut-brain axis. *Neuroscience*, 2013; 240: 287-296.
64. Salim S, Oxidative stress and the central nervous system. *J Pharmacol Exp Ther.*, 2017; 360: 201-205.
65. Muriach M, Flores-Bellver M, Romero FJ, Barcia JM, Diabetes and the brain: oxidative stress, inflammation, and autophagy. *Oxid Med Cell Longev.*, 2014; 2014: 1-9.
66. Silva YP, Bernardi A, Frozza RL, The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Front Endocrinol.*, 2020; 11: 25.
67. Robles-Vera I, Toral M, de la Visitación N, Aguilera-Sánchez N, Redondo JM, Duarte J, Protective effects of short-chain fatty acids on endothelial dysfunction induced by angiotensin II. *Front Physiol.*, 2020; 11: 277.
68. Amzăr AI, Udeanu DI, Pițuru MT, Hîrjău M, Popa DE, Velescu BS, Arsene AL, Signalling through the microbiota-gut-brain triade. *Farmacia*; 2022; 70(3): 402-409.
69. Yan F, Li N, Shi J, Li H, Yue Y, Jiao W, Wang N, Song Y, Huo G, Li B, *Lactobacillus acidophilus* alleviates type 2 diabetes by regulating hepatic glucose, lipid metabolism and gut microbiota in mice. *Food Funct.*, 2019;10(9): 5804-5815.