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ORIGINAL CONTRIBUTION

The novel insight into anti-inflammatory and anxiolytic effects of psychobiotics in diabetic rats: possible link between gut microbiota and brain regions

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Abstract

Purpose Type 2 diabetes mellitus (T2DM) was associated with gut microbial impairment (dysbiosis) and neurological and behavioral disorders. The role of the gut–brain axis in the management of many diseases including T2DM has been the focus of much research activity in the recent years. However, a wide knowledge gap exists about the gut microbial effects on the function of glia cells. Hence, the present study was aimed to examine the effects of psychobatics on dysbiosis and glia cells function in enteric and central nervous system with an inflammatory insight in T2DM.

Methods Thirty rats were treated by *Lactobacillus* (*L.*) *plantarum*, inulin, or their combination (synbiotic) for 8 weeks after inducing T2DM. Fecal sample was collected to evaluate gut microbial composition. Then, the rats were sacrificed, and the colon, amygdala, and prefrontal cortex (PFC) were studied.

Results T2DM resulted in dysbiosis and increased levels of glial cell-derived neurotrophic factor (GDNF), glial fibrillary acidic protein (GFAP), and inflammatory markers (IL-17, IL-6, and TLR-2) in the colon and brain. However, concurrent supplementation of *L. plantarum* and inulin could improve the gut microbial composition as well as reduce the levels of inflammatory cytokines. While the administration of *L. plantarum* led to a significant decrease in TLR-2 as well as GDNF and GFAP only in the amygdala, the synbiotic intake could make such changes in the colon, amygdala, and PFC.

Conclusions Our findings demonstrated an innovative approach to the beneficial effects of psychobiotics in neuroinflammation and behavioral performance through gut microbiota changes, focusing on possible role of glial cells in gut–brain axis.

Keywords Gut–brain axis · Anxiety-like behaviors · Glial cell-derived neurotrophic factor · Glial fibrillary acidic protein · Inflammation · Psychobiotic

Elaheh-Sadat Hosseinifard and Mohammad Morshedi have contributed equally to this work.

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Abbreviations

GDNF	Glial cell-derived neurotrophic factor
GFAP	Glial fibrillary acidic protein
IL	Interleukin
HFD	High-fat diet
LPS	Lipopolysaccharides
EPM	Elevated plus maze
TLRs	Toll-like receptors

Introduction

Type 2 diabetes mellitus (T2DM), as a metabolic disorder, is accompanied with low-grade inflammation [1]. Central neuropathy, as one of the most important complications of T2DM, can lead to functional and structural impairments of brain regions [2, 3].

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Today, the role of gut microbiota has been demonstrated in the pathogenesis of many diseases including obesity, T2DM, and neurological diseases [4]. Gut microbiota can be imbalanced (dysbiosis) by dietary and environmental factors as well as diseases [5, 6]. For instance, the decreased population of *Firmicutes* bacteria has been reported in diabetic rats [7].

Recent attention has been paid to the bidirectional relationship between the intestine and the brain (gut–brain axis). It was shown that gut dysbiosis and behavioral disorders can impact on each other [8]. Evidence indicates that increased population of *Bacteroidia* and *Clostridia* in T2DM can lead to a significant elevation of intestinal lipopolysaccharides (LPS) and subsequent systemic inflammation, interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) via the increased expression of Toll-like receptors (TLRs) [9, 10]. Neuroinflammation defined by IL-6, IL-17A, and TNF-α has been reported in glial cells, following the increased levels of LPS [11]. However, the exact mechanism of glia cell function in relation to gut microbial impairment is unclear.

Neuroinflammation can lead to neurological damage and behavioral disorders [12]. Recently, the role of glia cells (microglias and astrocytes) in neuroinflammation and mood disorders is documented [8]. Evidence shows that structure and function of the astrocytes are impaired in T2DM [13]. In this regard, glial fibrillary acidic protein (GFAP), an indicator of astrocytes activity, is altered in central nervous system (CNS) in diabetes which follows CNS disorders [14, 15].

Neurotropic factors such as glial cell-derived neurotrophic factor (GDNF) are required for the development, maintenance, and survival of neurons [16]. GDNF can reduce the level of inflammatory cytokines such as NF-κB [17]. Studies examining changes in the levels of GDNF and GFAP in both CNS and ENS in type 1 and 2 diabetes are limited and conflicting [18, 19], but their increased levels have been observed in inflammatory and neurological diseases. Functional impairment in various brain regions, including the hypothalamus, hippocampus, prefrontal cortex (PFC,) and amygdala, have been reported in T2DM [20–22]. On the other hand, there is some evidence that the interaction of the PFC with amygdala can modulate anxiety-like behaviors [23, 24].

Psychobiotics refer to beneficial bacteria (probiotics) or indigestible substances such as inulin (prebiotics) which can enhance the performance of the CNS and the behavioral mechanisms through interactions with the gut bacteria [22]. Probiotic administration was found to improve dysbiosis and reduce inflammation in obesity and insulin resistance in mice [25]. Administration of probiotics (*L. casei* and *L. plantarum)* could ameliorate neuroinflammation and behavioral disorder in rats with encephalopathy [26]. In addition, anxiolytic and anti-inflammatory effects of prebiotics have been observed previously, indicating that prebiotics can have a potential role in the treatment of psychiatric disorders [27, 28]. A study showed the beneficial effects of *Lactobacillus sporogenes* and inulin on antioxidant capacity and serum insulin levels in patients with diabetes [29]. Therefore, the present study was conducted for the first time to evaluate the effects of psychobiotics (*L. plantarum* and inulin) on the gut microbiota composition as well as GDNF and GFAP concentrations and their inflammatory responses both in the CNS and ENS of type 2 diabetic rats. Finally, the correlations between the study parameters and anxiety-like behavior were investigated.

Materials and methods

Animals

All rat experiments were carried out under Animal Experimentation Ethics Committee of Tabriz University of Medical Sciences (TBZMED) in accordance with the guidelines of the Principles of Laboratory Animal Care (NIH Publication, revised 1996) with approved license. Male Wistar rats (200 ± 20 g and 6 ± 1 week, $n = 30$) were obtained from Laboratory Animal Center of TBZMED, Iran. The rats were housed under controlled temperature (22–25 °C), humidity ($55 \pm 5\%$), and a 12 h: 12 h light/dark cycle with *ad libitum* access to drinking water.

Diets and T2DM induction

The healthy control group was fed a normal diet. To induce T2DM, the other groups were fed a high-fat diet (HFD), containing 58% fat for 4 weeks [30]. Then, T2DM was induced by a single intraperitoneal (IP) injection of streptozotocin (STZ, 35 mg/kg BW). Three days after injection, rats with fasting blood glucose level of ≥ 250 mg/dL were considered to be diabetic. The rats were kept on normal diet containing 12% fat, 22% protein, and 66% carbohydrate, after diabetes induction. The experimental rats were divided into five groups (6 rats per group), as below: Healthy Control (HC); Diabetic + *L. plantarum* (DL); Diabetic + inulin (DI); Diabetic + *L. plantarum* + inulin (DLI), and Diabetic Sham (DSh).

Supplementation

Lactobacillus plantarum ATCC 8014 was inoculated and cultured in Man–Rogosa–Sharpe (MRS) broth in aerobic conditions. A concentration of 10^7 colony-forming units (CFU)/mL of bacterial suspensions was freshly prepared within 8 weeks. Each rat was gastric gavaged every 24 h for 8 weeks [7]. Inulin, dissolved in drinking water, was calculated based on 5% of daily food intake.

Elevated plus maze (EPM)

EPM test was used to evaluate anxiety-like behavior [31]. The maze was located inside a sound-attenuated room. The apparatus was made of wood and consisted of Central Square (10 × 10 cm) with two open and two close arms (50 × 10 cm). All the evaluated parameters were recorded manually during the 5-min session by video camera, coupled to a computer.

Blood and tissue samples preparation

Rats were sacrificed after anesthetization with IP sodium pentobarbital (Sigma, 65 mg/kg BW); amygdala, PFC, and colon were removed. Colon tissues were cleaned of their contents with cold saline. The tissues were homogenized and centrifuged at 9000 rpm at 4 °C for 10 min [22].

Next-generation sequencing (NGS)

The complete details of the method of metagenomics performance are explained in the supplementary section (supplementary). Briefly, stool samples were collected and stored at − 80 °C. DNA extraction was carried out. Then, 16S rRNA gene amplicon library was prepared and high-throughput sequencing was done. Finally, the sequences were analyzed, using the QIIME 1.6.0 software.

Biochemical tests

The concentrations of GDNF, GFAP, TLR-2, IL-17A, and IL-6 in the supernatants of both colon and brain tissues were determined, using enzyme-linked immunosorbent assay (ELISA) kits, normalized by protein content, according to the manufacturer's instructions (Bioassay Technology Laboratory).

Statistical analysis

Statistical analysis was performed through SPSS 23. Comparisons of quantitative variables were carried out by one-way ANOVA, followed by Tukey's post hoc test. The Pearson's bivariate correlation coefficient was used to check correlations between parameters. Data were expressed as means ± standard error (SEM) for continues variables. For all statistical tests, significance was set at $P < 0.05$.

Results

1. The gut–microbiota composition.

The results of metagenomics of fecal microbiota showed that dramatic changes in the gut bacterial composition and population of the diabetic vs. healthy rats were observed. Composition of the microorganisms (domain) of each group is shown in Fig. 1 which indicates no significant changes in the DSh, compared to the HC group. The evaluation of class-based bacterial groups A showed that while the population of *Bacteroidia* and *Clostridia* bacteria (Fig. 2) was found to increase significantly, those of *Bacilli* decreased in the DSh, compared to the HC group. However, significant increases in the *Bacilli* population were observed in the DI (29.31%), DL (57.38%), and DLI (80.497%) groups, after supplementation (Fig. 2). The decrease rate in the population of *Bacteroidia* in the diabetic rats was more than 40%, following the intake of the synbiotic. The highest percentage of *Actinobacteria* was associated with inulin (DI) intake, compared to the HC group (Fig. 2). Concerning the five bacterial species with the highest frequency in each group, it was observed that, in the DSh group, predominant populations were *Prevotella* species, while, in the other study groups, the dominant populations were *lactobacillus* ones (Fig. 3).

Considering eight categories of order-based bacteria, there was a dramatic change in the population and composition of the microbiota in the diabetic rats, compared with the HC group. Most species of the population in the DSh group were *Prevotella oris* and *Ruminococcus flavefaciens*, while the treated groups mostly consisted of *Lactobacillus* species (Fig. 4). After supplementation, the growth of *Lactobacillales* was increased in the DI, DL, and DLI groups (29.08%, 57.33%, and 78.64%), respectively (Fig. 4). By calculation of the important proportions of the bacterial population, significant changes were also found in the intervention groups, compared to the DSh group (Fig. 5). In the DLI group, the relative abundance of *Lactobacillales* was increased in comparison with *Bacteroidales* [DLI (21.76%), DL (1.78%), and DI (0.84%)]; in addition, the population of *Clostridiales* was increased [DLI (2.37%), DL (7.28%), and DI (0.98%)]. Moreover, greater ratio of *Bacteroidales*/*Firmicutes* (0.966%) and *Clostridiales*/*Firmicutes* (0.65%) was observed in the diabetic rats, reversed by supplementation, especially synbiotic intake.

2. Levels of inflammatory markers in the amygdala and PFC.

Compared with HC group, a significant increase in the levels of IL-6 and IL-17A was found in the amygdala and PFC of the diabetic rats ($P < 0.001$) (Fig. 6). Moreover, there was a significantly increased TLR-2 levels in the amygdala ($P < 0.001$) and PFC ($P = 0.001$), compared to the HC group. Treatment with all three psychobiotics (*L. plantarum*, inulin, and their combination) led to the decreased levels of inflammatory markers in the diabetic rats, compared with the DSh group. Eight weeks of treatment with *L. plantarum* also led to a significant reduction in the levels of IL-17A ($P = 0.040$, $P = 0.029$) and IL-6 ($P = 0.006$, $P = 0.013$) in the amygdala and PFC, compared to the DSh group, respectively. Inulin

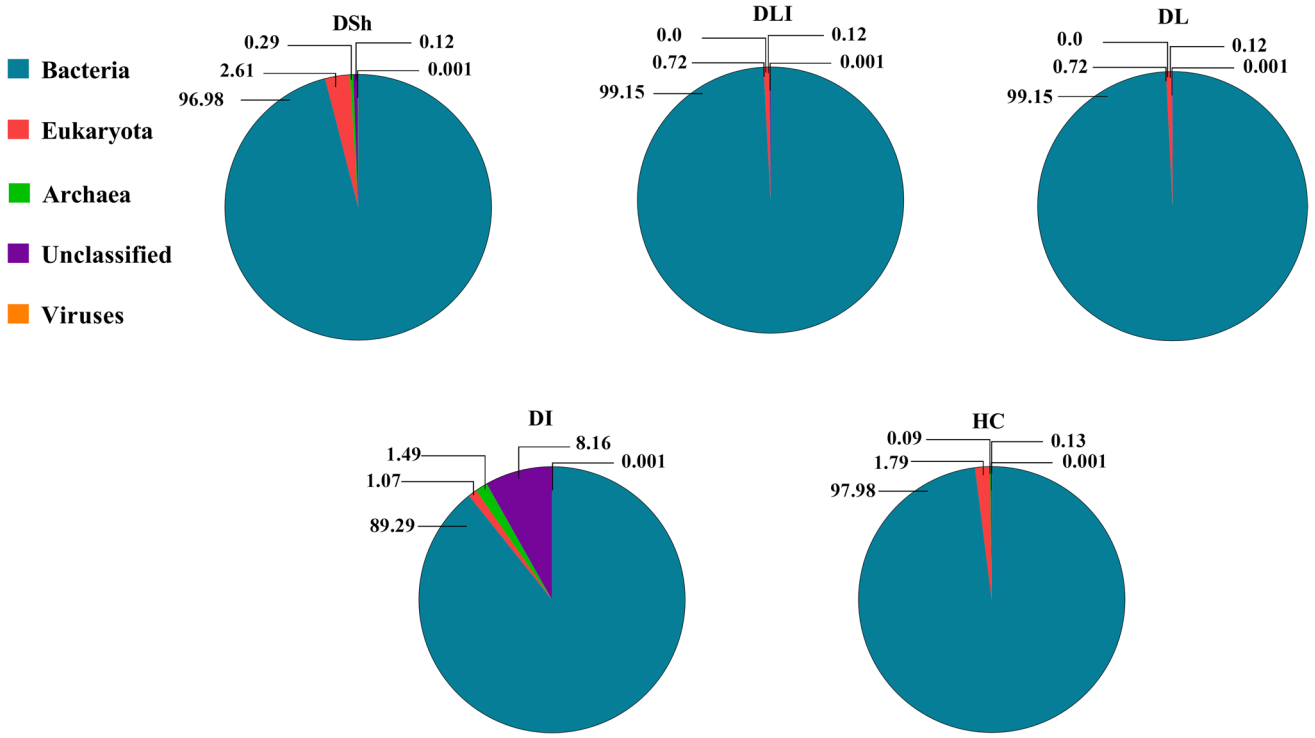

Fig. 1 Aggregate microbiota composition at domain level in fecal samples from healthy and diabetic rats. *HC* healthy control, *DLI* diabetics treated by the *L. plantarum* and inulin, *DI* diabetics treated by inulin, *DL* diabetics treated by the *L. plantarum, DSh* diabetic Sham. Repeated-measures analysis was used. Data were expressed as relative abundance

consumption only reduced the levels of IL-6 ($P=0.041$) in the amygdala, compared with the DSh group; however, no significant change was observed in the other inflammatory markers in the amygdala and PFC in the DI group. In addition, the use of synbiotic agents improved the concentrations of IL-6 ($P<0.001$ and $P=0.008$) and IL-17A ($P=0.012$ and $P=0.006$) in the amygdala and PFC, compared to the DSh group, respectively. TLR-2 levels decreased significantly only in the amygdala (not PFC) in the DLI group, compared to the DSh group ($P=0.007$). It should be noted that supplementation with synbiotic also showed a significant decrease in IL-6 levels in the amygdala, compared to the DI group ($P=0.047$).

3. Levels of inflammatory markers in the colon.

The levels of all three colonic inflammatory markers including IL-6, IL-17A, and TLR-2 were significantly increased in the DSh group, compared to the HC group (Fig. 6). In contrast, a significant decrease in IL-17A and IL-6, as inflammatory factors of colon, was observed in the DL ($P=0.006$, $P=0.007$) and DI ($P=0.031$, $P=0.047$) groups, respectively. *Lactobacillus plantarum* and inulin alone did not reduce TLR-2 levels in the colon. Synbiotic intake resulted in a reduction in the levels of colonic TLR-2 ($P=0.029$), IL-6 ($P<0.001$), and IL-17A ($P=0.002$) in the DSh group. There was no significant difference among the treatment groups.

4. Levels of GFAP and GDNF in the amygdala and PFC.

Increased concentrations of GFAP ($P<0.001$, $P=0.007$) and GDNF ($P<0.001$, $P=0.003$) in the amygdale and PFC of the diabetic rats were observed, in comparison to the HC group (Fig. 7). Inulin consumption alone could not significantly alter GDNF and GFAP levels in any of the brain regions, compared to the DSh group. Supplementation with the *L. plantarum* led to a reduction in GFAP ($P=0.014$) and GDNF ($P=0.033$) levels only in the amygdala; however, these changes were not significant in the PFC, compared to the DSh group. The intake of synbiotics significantly reduced GFAP and GDNF levels in the amygdala ($P=0.004$). In addition, compared to the DSh group, the reduction of GFAP ($P=0.027$) but not GDNF levels in the PFC was followed by synbiotics consumption. There was no significant difference among the intervention groups.

5. Levels of GFAP and GDNF in the colon.

Diabetes led to an increase in the levels of GFAP and GDNF in the colon, compared to the healthy rats (Fig. 7). The separate supplementation of inulin and the *L. plantarum* could not reduce the levels of GDNF and GFAP

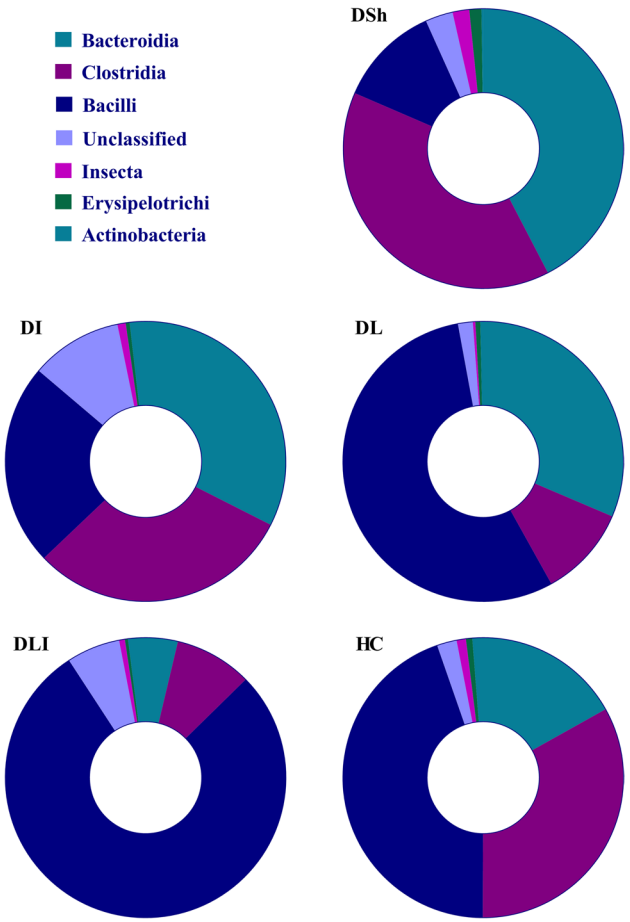

Fig. 2 The effect of the *L. plantarum*, inulin, and their combination intake on the composition and diversity of gut microbiota (Class assignment). *HC* healthy control, *DLI* diabetics treated by the *L. plantarum* and inulin, *DI* diabetics treated by inulin, *DL* diabetics treated by the *L. plantarum, DSh* diabetic Sham. Repeated-measures analysis was used. Data were expressed as relative abundance

in the colon, compared to the DSh group, while the synbiotic administration could significantly decrease GFAP ($P = 0.008$) and GDNF ($P = 0.036$) levels. No significant difference was observed among the treatment groups.

6. EPM.

The results showed that the percentage of total time spent in open arm by diabetic sham rats was lower in comparison with the HC group ($P < 0.001$); however, it was higher than all three intervention groups. Post hoc comparison using Tukey's test showed that the open/closed arm duration ratio in the DSh group was lower than the DL, DI, and DLI groups ($P < 0.001$). In addition, there was no significant difference among the treatment groups (Fig. 8).

Discussion

The present study for the first time showed that an 8 week supplementation with the *L. plantarum* and inulin and especially their combination could reverse the changes developed by T2DM in the gut microbial population and composition, as well as in the levels of TLR-2, IL-6, and IL-17, and glia cells function (GDNF and GFAP) both in the colon and brain regions of the diabetic rats. In addition, it indicated a strong correlation of gut and brain parameters with anxiety which improved through normalization of the gut microbial composition, following supplementations.

One of the most important findings of the present study was the evaluation of the gut microbial changes, induced by T2DM. We showed that the daily consumption of the *L. plantarum* led to an increase in the population of *Lactobacillus* species which resulted in a decrease in the amount of the group of pathogens such as *Clostridiales, Desulfovibrionales, Selenomonadales*, and *Bacteroidales* (Figs. 3, 4). Compared to the administered probiotic, inulin had a weaker effect on the gut microbial shift in a healthy manner; however, when taken synbiotic, there was a significant impact on the growth of beneficial bacterial species (Figs. 2, 5), due to the synergistic effect of these two supplements on microbial changes.

In the present study, T2DM caused the increased levels of TLR-2, IL-6, and IL-17 in the gut and brain. However, reduced levels of these parameters in the colon and brain were observed after the improvement of dysbiosis with our supplementation. In addition, there was a strong correlation among the levels of inflammatory markers in the amygdala, colon, and PFC; this association was stronger between the colon and the amygdala (Supplementary Table 1 and 2). On the other hand, dysbiosis was associated with the increased levels of inflammatory markers in the diabetic rats (Fig. 9). Inflammation is one of the major causes of neurological (CNS and ENS) and behavioral disorders, and plays an important role in diabetes complications [1, 32, 33]. We also found a positive correlation between anxiety and inflammatory markers in colon and brain (supplementary Table 3). Consistent with our findings, Li et al. [7] showed that *L. casei* CCFM419 (8×10^{10}) consumption for 10 weeks resulted in the improved microbial composition that was accompanied by reduction in serum IL-6 and TNF-α as well as increased IL-10 in mice with T2DM. The pathogenic bacteria can also pass through blood–brain barrier)BBB) and through their receptors on the brain cells (neurons and glia cells) which consequence is neuroinflammation [34].

In this study, though the use of the mere *L. plantarum* or inulin decreased IL-6 and IL-17A, the reduction of TLR-2

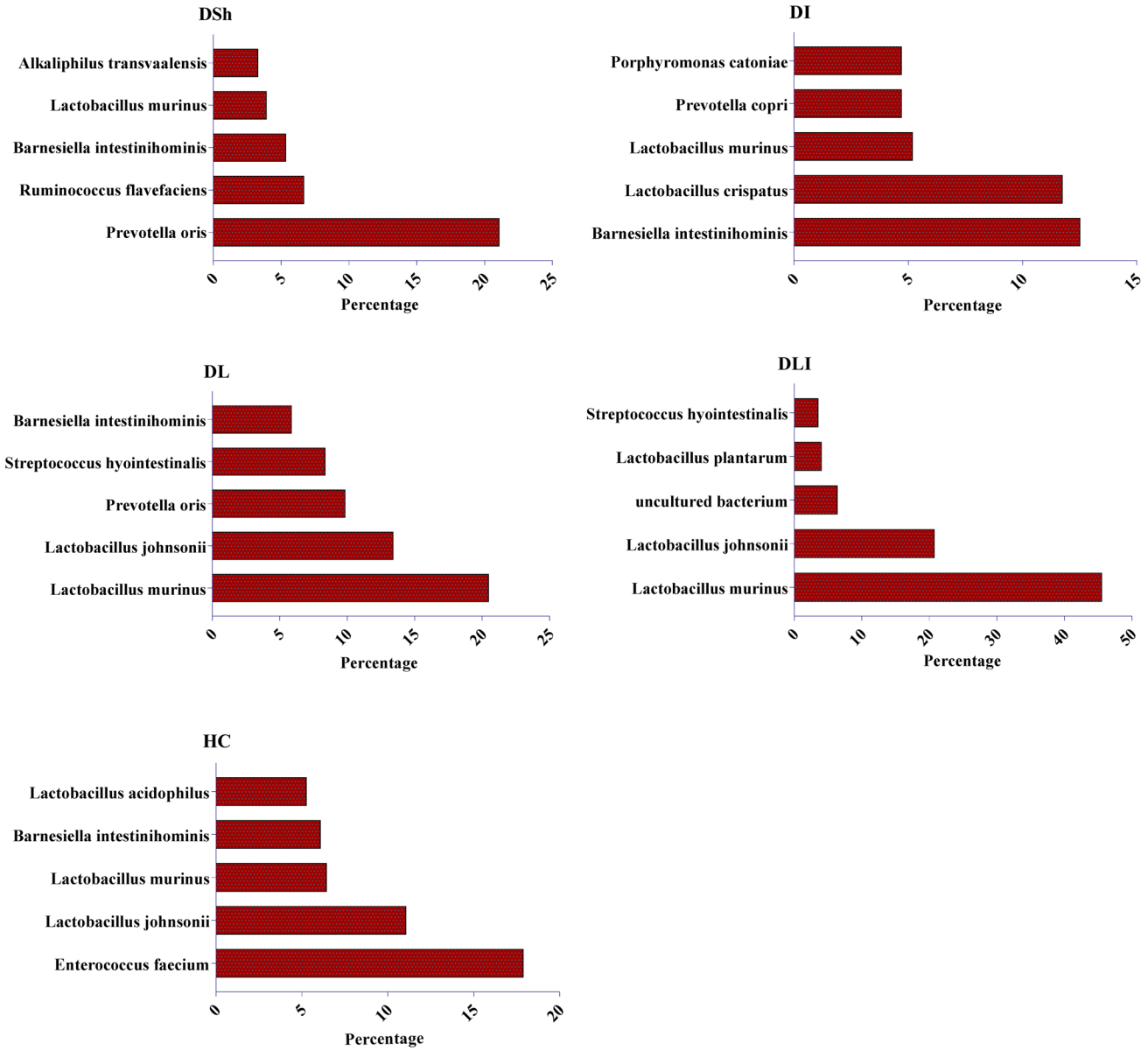

Fig. 3 Variation in the proportion of five most populated species in fecal samples. *HC* healthy control, *DLI* diabetics treated by the *L. plantarum* and inulin, *DI* diabetics treated by inulin, *DL* diabetics treated by the *L. plantarum, DSh* diabetic Sham. Repeated-measures analysis was used. Data were expressed as relative abundance

levels in the colon, PFC, and amygdala did not reach the significant level. It seems that these supplements might have decreased inflammation by reducing the other TLRs activities like TLR-4 which is one of the most important TLRs in identifying pathogen-associated molecular patterns (PAMPs) [35]. In addition, the other factors such as control of blood glucose and reduction of oxidative stress in the serum and brain, as reported in our previous work, can have an important role in reducing inflammation [22, 36]. Moreover, since TLR-2 is reported to recognize lipoprotein from Gram-positive bacteria [35], it might be reactivated by the *L. Plantarum* supplementation, despite the gut microbial improvement. Antonietta Rizzo et al. [35] showed that the use of *L. plantarum* in healthy rats could increase TLR-2 levels in comparison with the control group. It should also be noted that the activation of TLR-2, besides the production of inflammatory markers, exerts cytoprotective effects [37]. TLRs are also expressed in the CNS and involved in nervous system development, including ENS homeostasis, which among all TLRs; TLR-2 appears to be a major player in gut homeostasis. In a study, oral chitosan oligosaccharides (200, 400, or 800 mg/kg) consumption had neuroprotective effects on rats with Alzheimer via inhibiting oxidative stress and

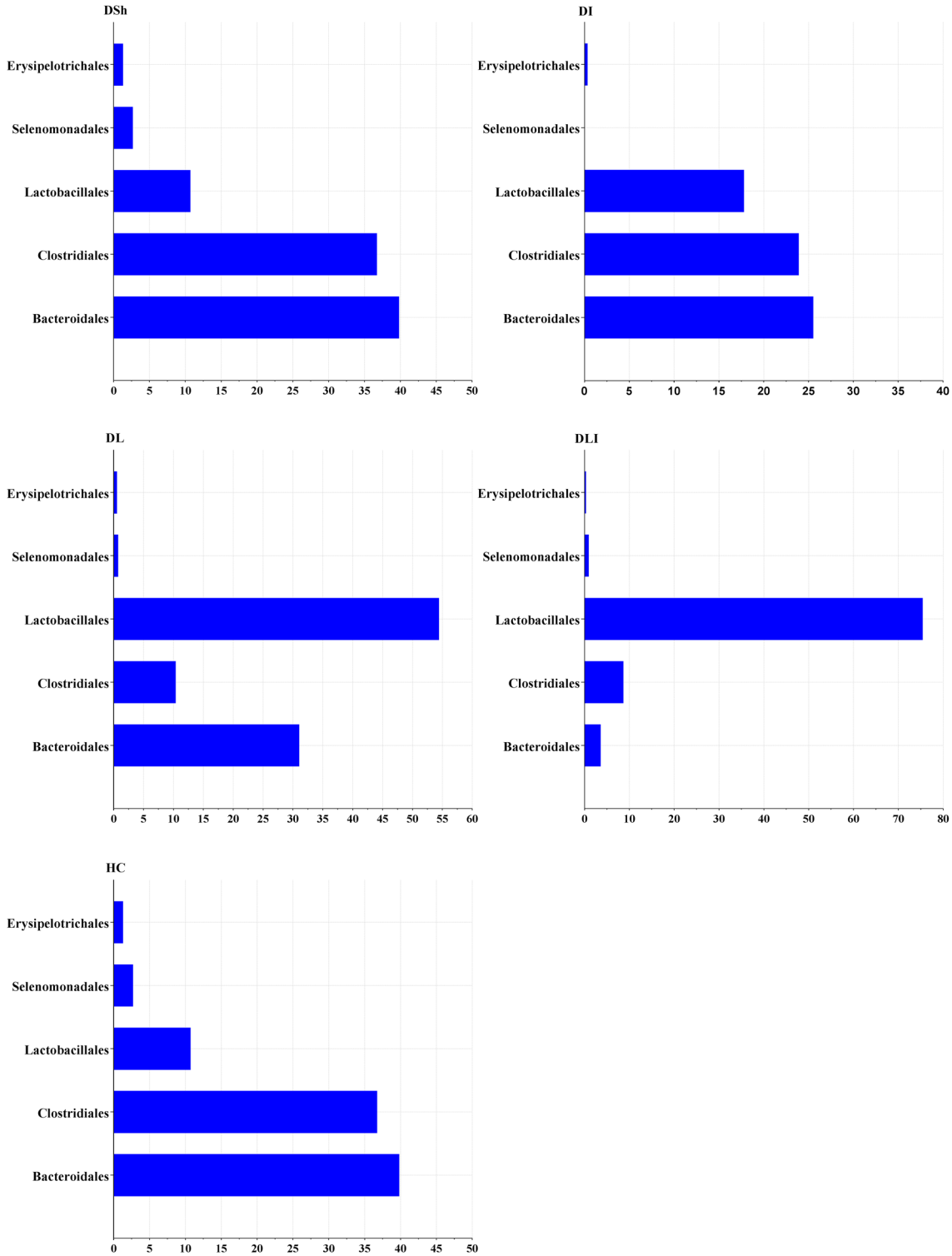

Fig. 4 Major taxonomic level range from order base on metagenomics data. *HC* healthy control, *DLI* diabetics treated by the *L. plantarum* and inulin, *DI* diabetics treated by inulin, *DL* diabetics treated by the *L. plantarum, DSh* diabetic Sham. Repeated-measures analysis was used. Data were expressed as relative abundance

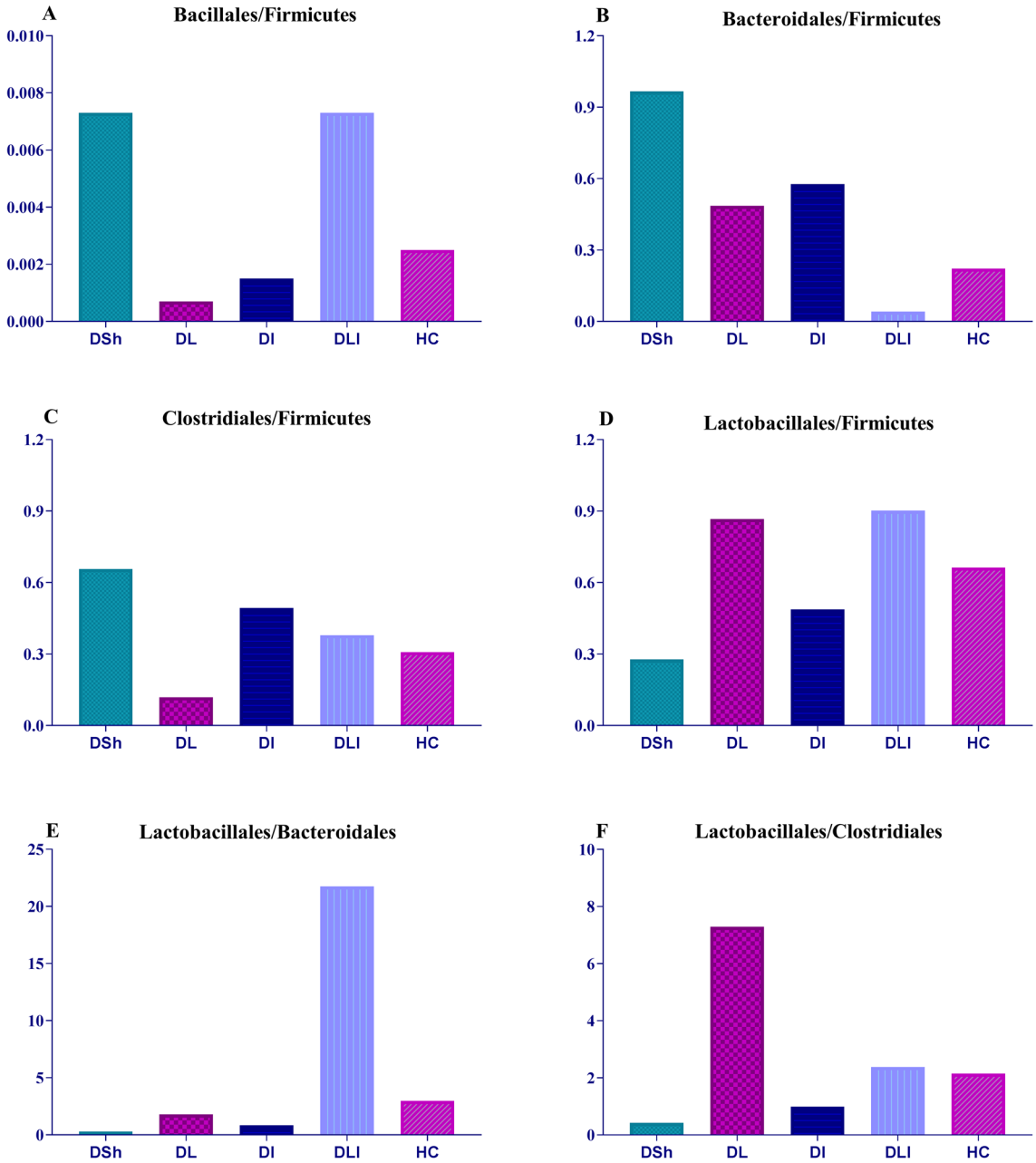

Fig. 5 **a**–**f** The most important ratios based on metagenomics data in fecal samples. *HC* healthy control, *DLI* diabetics treated by the *L. plantarum* and inulin, *DI* diabetics treated by inulin, *DL* diabetics treated by the *L. plantarum, DSh* diabetic Sham. Repeated-measures analysis was used. Data were expressed as relative abundance

neuroinflammatory responses (i.e., reducing hippocampal IL-1β and TNF-α). The levels of inflammatory markers increased more in the amygdala than the colon, suggesting that the amygdala is more vulnerable than either the colon or PFC in T2DM.

In the present work, increased levels of GDNF and GFAP were observed in the colon and brain regions of the diabetic rats. Elevation of these markers in the colon and brain can be due to dysbiosis and increased inflammation developed by T2DM, which, with the compensatory mechanism, activates glial cells to prevent nerve damage and support neurons. We also demonstrated that there was a close relationship between the amount of *Lactobacillales* and *Clostridiales* bacteria with the concentration of GFAP (Fig. 9). The increase of GDNF in mice with T2DM may occur through increasing TLR-2 (GDNF-TLR-2 axis) [37]. Contrary to our results, in a study by Wei Liu et al. [19], a decrease in GDNF and GFAP expression was reported in ENS of type 1 diabetic

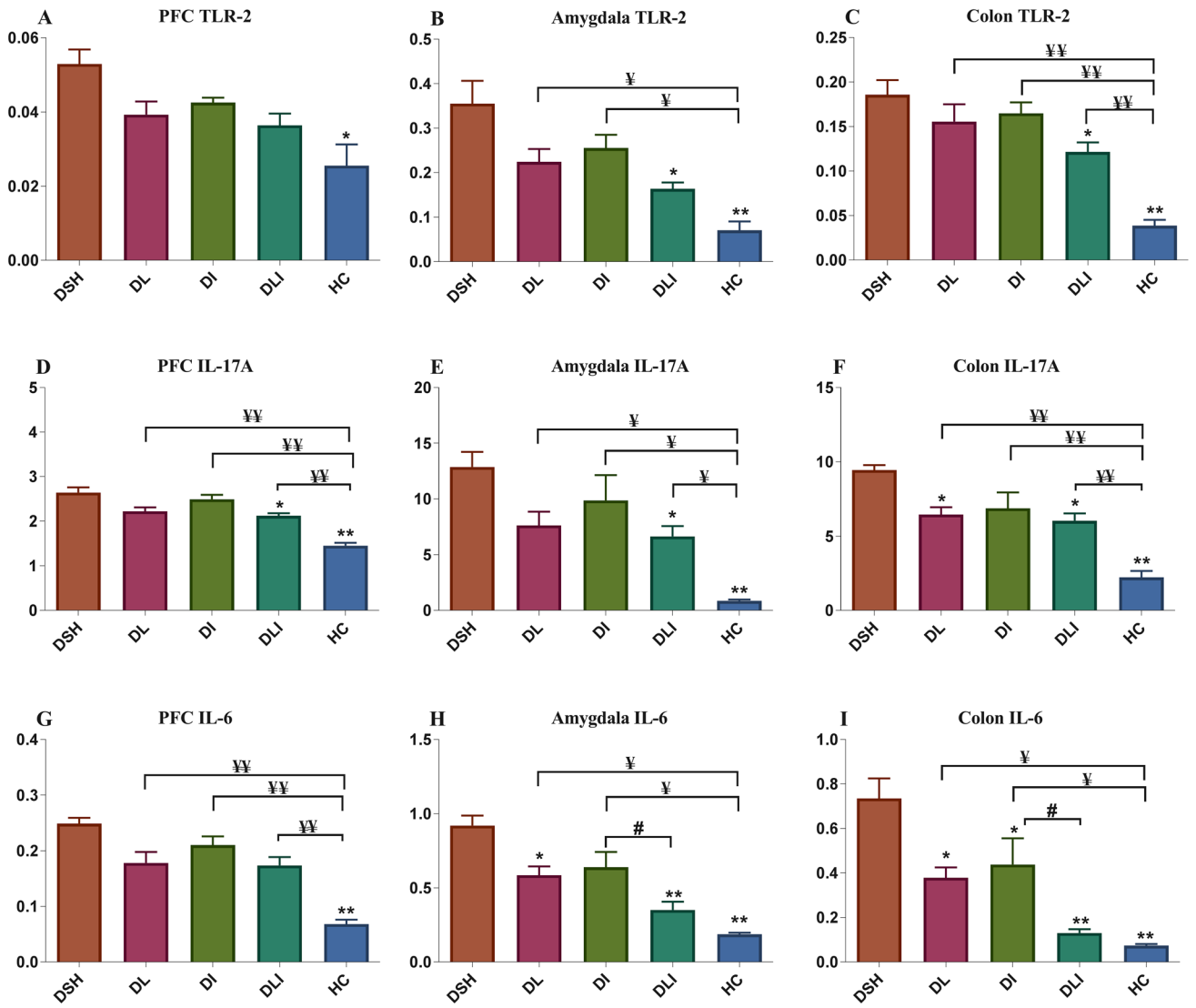

Fig. 6 Effects of the *L. plantarum* and inulin on levels of inflammatory markers in the control and diabetic rats ($n=30$). **a–c** The level of TLR-2, (D-F) IL-17, and (G, I) IL-6 in the PFC, amygdale, and colon of the control and diabetic rats, respectively. *HC* healthy control, *DSh* diabetic sham, *DLI* diabetics treated by the *L. plantarum* and inulin, *DL* diabetics treated by the *L. plantarum, DI* diabetics treated by inulin. $^{*}P<0.05$ and $^{**}P<0.001$ in comparison with DSh group. $^{¥}P<0.05$ and $^{¥¥}P<0.001$ in comparison with the HC group. $^{\#}P<0.05$ in comparison with intervention groups. One-way analysis of variance, followed by post hoc Tukey's tests, was used. Data were expressed as mean ± SEM and $P<0.05$ was regarded as statistically significant

rats, while, in a study on rats with T2DM, increased levels of GFAP have been observed in spinal cord.

The astrocytes play an important role in supplying glucose and its metabolites to the neuron [13]. In the present research, a strong correlation was found between hyperglycemia and GFAP levels (Fig. 10). Therefore, any change in blood glucose levels can make significant changes in the function of the astrocytes [13]. In general, studies investigating the effects of T2DM on GDNF are limited. However, increase in GDNF production has been observed in cases of neurodegenerative conditions, including aging and Alzheimer's [38, 39]. In a study, LPS injection increased the expression of GDNF in the CNS, but the expression of GDNF receptors declined [40]. Ya Jin et al. [18] also reported that GDNF and GFAP levels were increased in the infants of mice with gestational diabetes; they indicated that this is due to the increased production of glial cells through the exposure of mice to maternal hyperglycemia.

The results of the present study also indicated that the use of synbiotic led to a decrease in GDNF and GFAP levels in both ENS and CNS. In a study [41], administration of *L. Fermentum* reduced the hyperactivity of astrocytes and decreased GFAP expression in normal growing rats. Studies

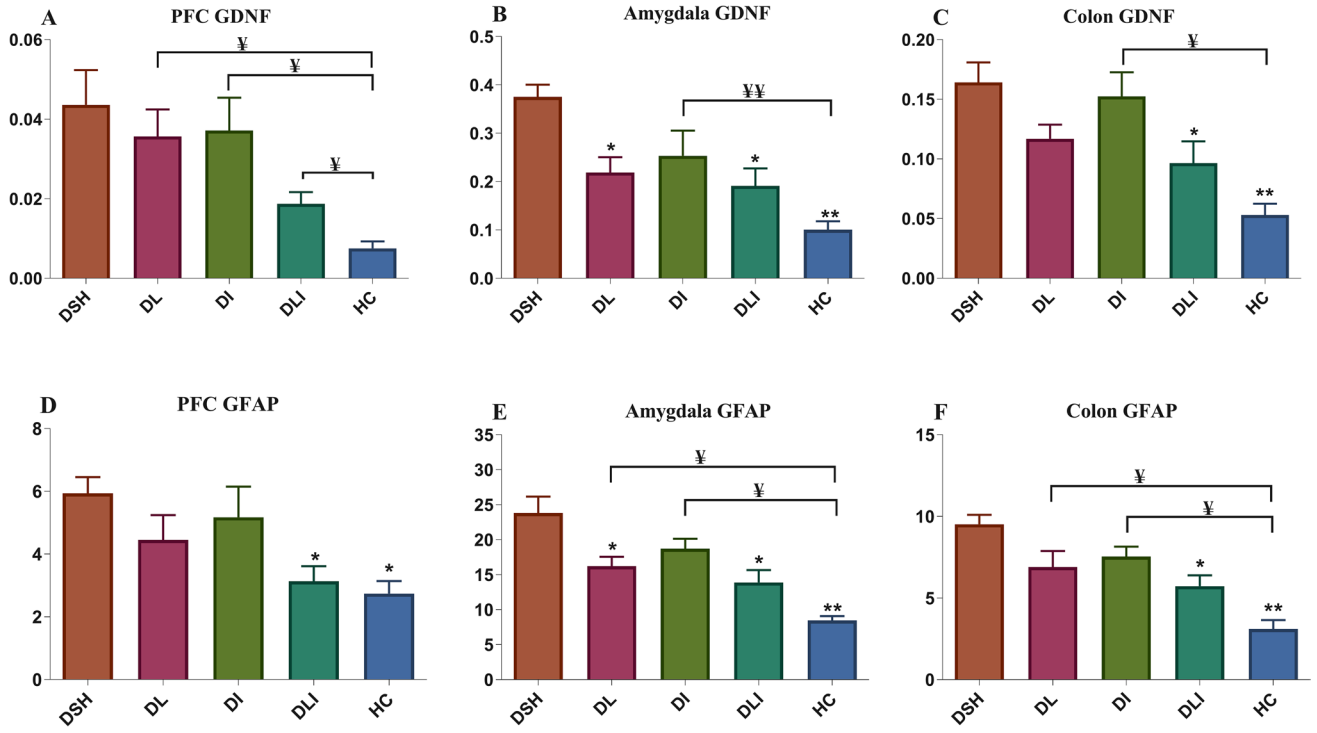

Fig. 7 Effects of the *L. plantarum* and inulin on GDNF and GFAP levels in ENS and CNS of the control and diabetic rats ($n=30$). **a–c** The level of GDNF and **d–f** GFAP in the PFC, amygdale, and colon of the control and diabetic rats, respectively. *HC* healthy control, *DSh* diabetic sham, *DLI* diabetics treated by the *L. plantarum* and inulin, *DL* diabetics treated by the *L. plantarum, DI* diabetics treated by inulin. $^{*}P<0.05$ and $^{**}P<0.001$ in comparison with DSh group. $^{¥}P<0.05$ and $^{¥¥}P<0.001$ in comparison with the HC group. One-way analysis of variance, followed by post hoc Tukey's tests, was used. Data were expressed as mean ± SEM and $P<0.05$ was regarded as statistically significant

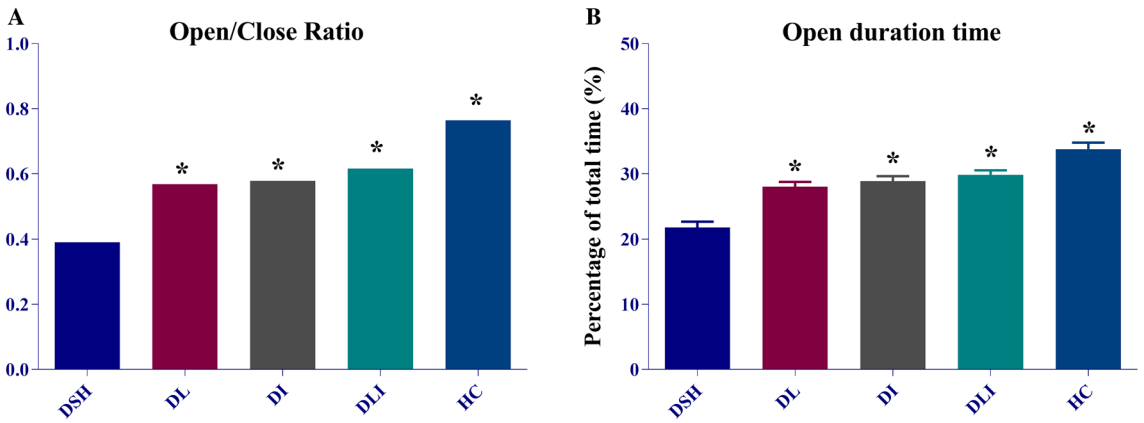

Fig. 8 Effect of the *L. plantarum* and inulin on anxiety-like behavior in the elevated plus maze test in the control and diabetic rats ($n=30$). **a** The ratio of the time elapsed in the open arms to the closed arms. **b** Percentage of time spent in the open arms. *HC* healthy control, *DSh* diabetic sham, *DLI* diabetics treated by the *L. plantarum* and inulin, *DL* diabetics treated by the *L. plantarum, DI* diabetics treated by inulin. $^{*}P<0.05$ in comparison with DSh group. One-way analysis of variance, followed by post hoc Tukey's tests, was used. Data were expressed as means ± SEM. $P<0.05$ was regarded as statistically significant

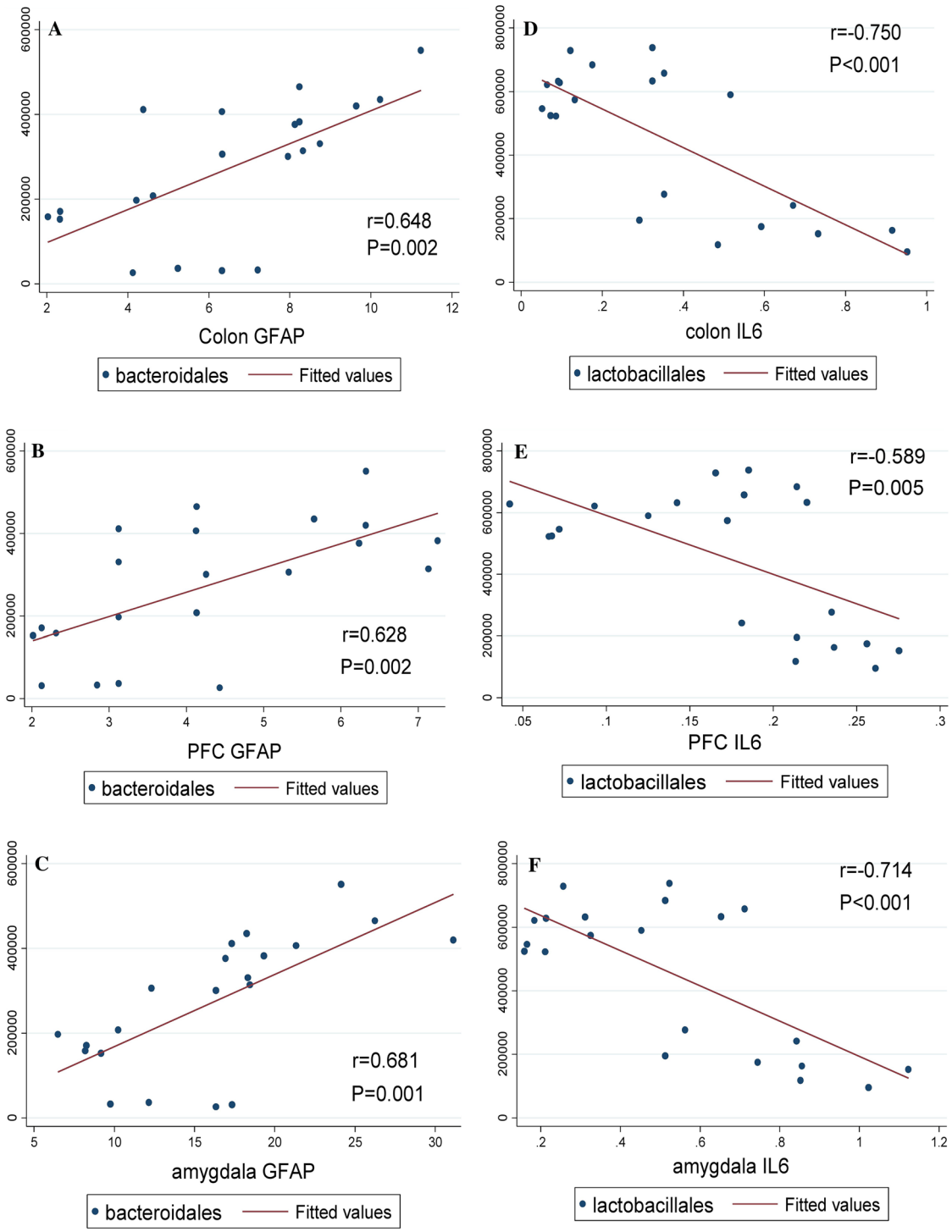

Fig. 9 Correlation coefficients between gut microbiome population with the levels of parameters in colon and brain. **a, b** Correlation of bacteroidales with GFAP in colon (**a**), PFC (**b**), and amygdala (**c**). **d–f** Correlation of lactobacillales with IL-6 in colon (**d**), PFC (**e**), and amygdala (**f**) of the control and diabetic rats

investigating the effects of gut microbiota on brain and glia cells are very confined.

Our results were also suggestive of anxiolytic properties of psychobiotics in diabetic rats (Fig. 8). In addition, we showed that there was a close association between the increase in *Lactobacillales* bacteria and the decrease in anxiety (Fig. 11). Evidence demonstrated that gut dysbiosis, hyperglycemia, and anxiety can have important effects

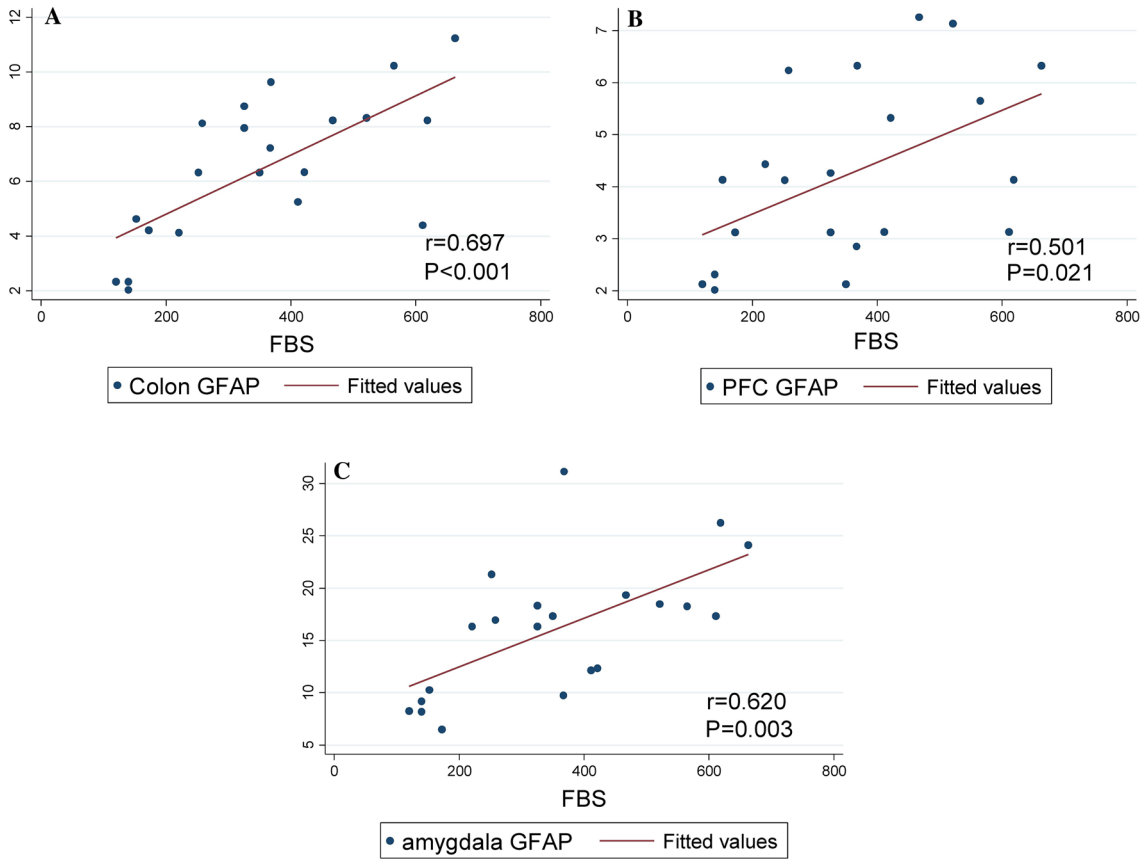

Fig. 10 Correlation coefficients between levels of fasting blood sugar and GFAP levels in the colon (**a**), PFC (**b**), and amygdala (**c**) of the control and diabetic rats ($n=30$). Correlations between two variables were computed by Pearson correlation coefficients. Data were expressed as means ± SEM. $P<0.05$ was regarded as statistically significant

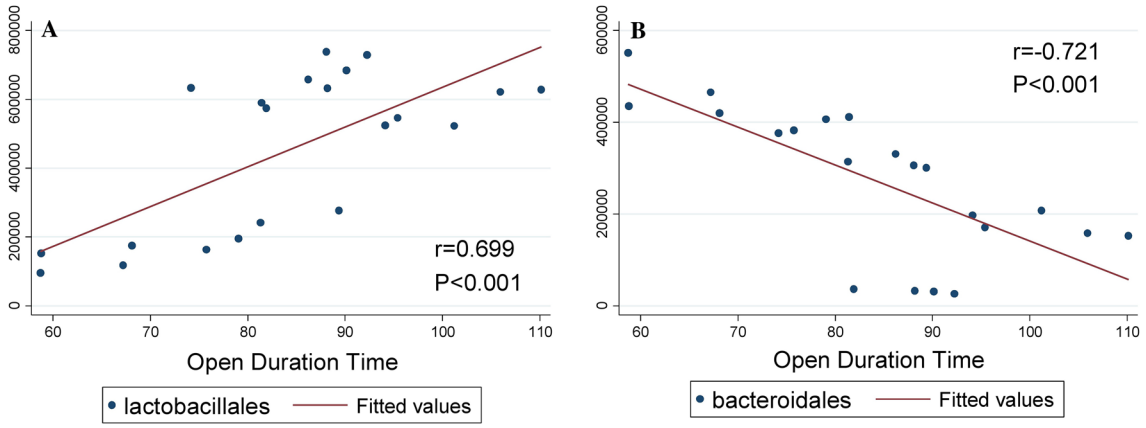

Fig. 11 Correlation coefficients between gut–microbiome populations with anxiety-like behavior. Correlation of **a** lactobacillales and **b** bacteroidales with open duration time

on each other [8]. Helene et al. [27] reported that administration of non-digestible galacto-oligosaccharides (GOS) for 3 weeks reduced cortical IL-1B and anxiety induced by LPS injection in mice. In addition to the elevated levels of anxiety as well as inflammatory markers, GDNF and GFAP in the present study, they strongly correlated with each other among the diabetic rats; these correlations were also strong between the two brain regions (supplementary Table 1, 2). Evidence shows that there is a structural and functional relationship between the amygdala and PFC; the

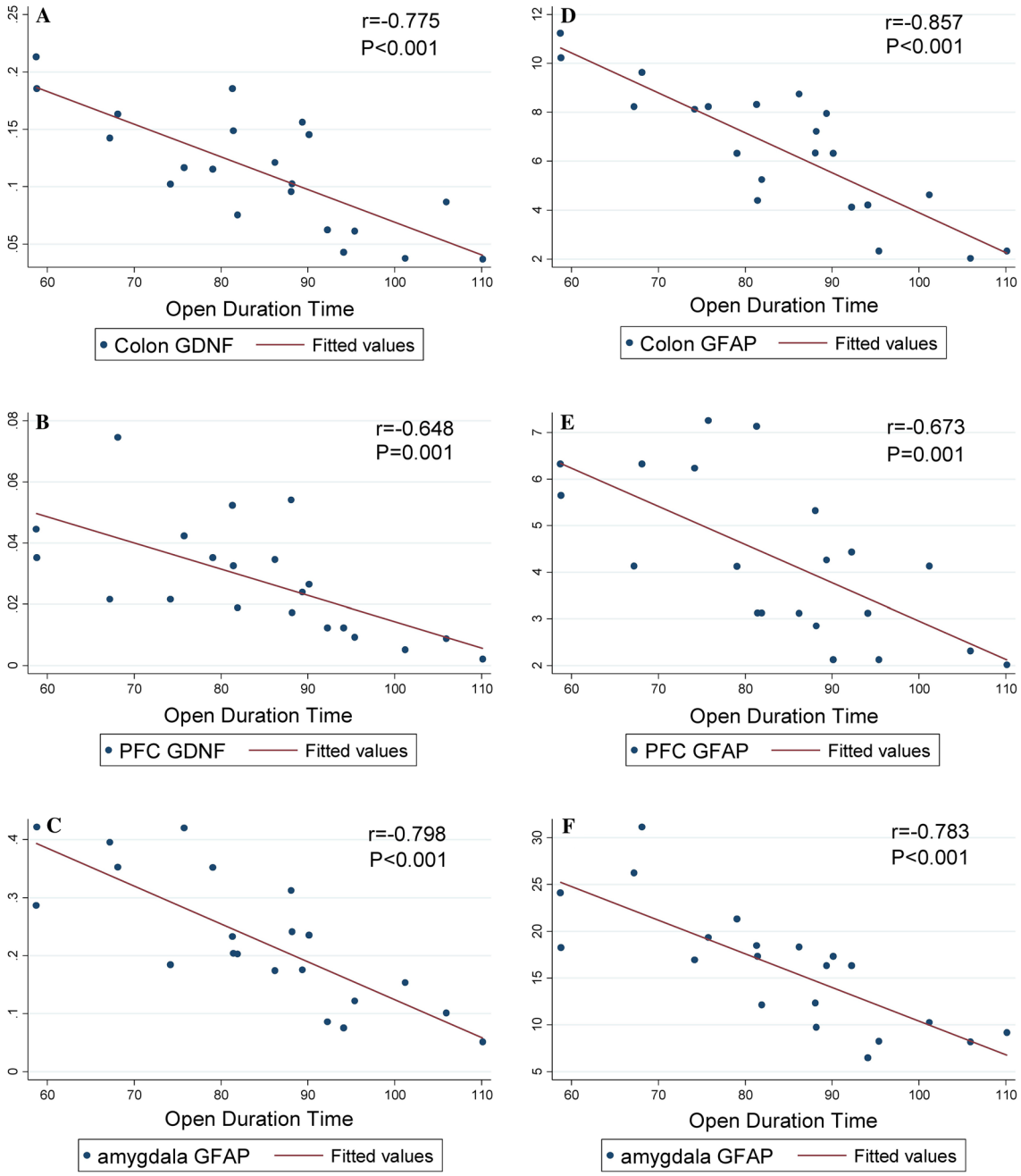

Fig. 12 Correlation coefficients of GDNF and GFAP levels with anxiety-like behavior in elevated plus maze test. Correlation of open duration time with **a**–**c** GDNF and **d**–**f** GFAP in the colon, and PFC and amygdala in the control and diabetic rats ($n=30$), respectively. Correlations between two variables were computed by Pearson correlation coefficients. Data were expressed as means ± SEM. $P<0.05$ was considered statistically significant

PFC has an inhibitory effect on the amygdala which control anxiety response [23, 24]. In the present work, we also observed a positive correlation of the GFAP and GDNF with anxiety-like behavior (Fig. 12). Burokas et al. [42] strongly suggested antidepressant and anxiolytic effects of prebiotics (FOS + GOS) in male mice [42, 43]. Studies have also shown that behavioral disorders have a direct correlation with production of inflammatory cytokines [44]. In a study by Morshedi et al. [22], the use of *L. plantarum* and inulin in diabetic rats improved depression and anxiety-like behavior through the alleviation of oxidative stress and increased levels of amygdale serotonin and BDNF concentration [22].

We investigated the effects of T2DM at the end of 8 weeks; however, it would be better to devise a study in

which the levels of these parameters are measured at different intervals. Since changes occurred in the first stages of diabetes induction might differ from those happened after the disease progression.

Conclusion

The present study showed that improving the gut microbiota composition led to decreased inflammation and anxiety after psychobiotics supplementation. In addition, it indicated that psychobiotics resulted in improved GDNF and GFAP levels in both gut and brain regions, especially in the amygdala region. According to the results, it seems that there is a possible link among the gut, amygdala, and PFC. This is the first study on the effect of psychobiotics on the gut–brain axis, opening new venues in the field of gut microbiota and glial cells' interaction. Therefore, further studies are needed to obtain more results.

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Author contributions MSA and ESH wrote the study design and protocol. MM and KBV helped with preparation of inulin and bacterial solutions and performing intervention phases. MM and ESH analyzed and interpreted the data and drew graphs. ESH and MM helped with keeping rats and intervening. ESH and MM performed behavioral tests and analyzed and interpreted the related data. ESH and MM and MSA were involved in drafting the manuscript or revising it critically for content. All authors have given the final approval of the version to be published.

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Compliance with ethical standards

Conflict of interest There is nothing to declare.

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