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Research Article

Assessment of Streptococcus *salivarius sp* thermophiles Antioxidant Efficiency and its Role in Reducing Paracetamol Hepatotoxicity

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Background: Probiotics have attracted a great attention aiming to develop natural non-toxic antioxidants, because of their role in decreasing the risk of reactive oxygen species (ROS) accumulation.
Objectives: The purpose of this study was to assess the antioxidant activity of a probiotic *Streptococcus salivarius* ssp *thermophillus* (St.sa) and to evaluate its protective effect against the oxidative stress induced by a toxic dose of paracetamol in Wistar rats.
Materials and Methods: Several assays were used to investigate the in vitro antioxidant capacity of the strain. To evaluate the protective effect against oxidative stress induced by paracetamol in liver, hepatic marker enzymes, the antioxidant enzyme activities, malondialdehyde (MDA) and glutathione (GSH) content in liver tissues were investigated.
Results: The strain has shown a considerable ability to scavenge DPPH free radical (89.43%),a good resistance to hydroxyl radicals (47%), a considerable ability to chelate iron ions (33.21%) and a good inhibitory effect against plasma lipid peroxidation (54.36%). Significant changes in liver function tests, antioxidant enzyme activities, MDA and GSH levels in paracetamol treated group were obtained compared to control group. Pretreatment with probiotic removed significantly the inhibition of antioxidant enzymes and suppressed MDA increase and GSH depletion. The analysis of the level of mRNA expression of antioxidant enzymes showed no significant differences in the expression of the enzymes in treated or non-treated groups.
Conclusion: This finding emphasizes the protective role of probiotics against ROS generated during the treatment with paracetamol.
Keywords: *Streptococcus salivarius*; paracetamol; hepatotoxicity; antioxidant activity; probiotic

1. Background

Smoking, environmental pollutants, radiations, chemicals and also drugs can lead to the production of free radicals (1). In a normal cell, there is a balance between formation and removal of free radicals. However, this balance can be shifted leading to disruption between the production of reactive oxygen and its antioxidant defense system because of either depletion of antioxidants or accumulation of reactive oxygen species (ROS), leading to a state called oxidative stress (1-2). It is well known that the intake of antioxidant elements protects cells from ROS effect and reduce the oxidative stress damages (3).

An increasing interest has been focused to develop natural nontoxic antioxidants, like probiotics that have attracted a great attention. One of the most recently adopted definition of probiotics cited that probiotics are defined as organisms that confer health benefit to consumers when administered in adequate amount (4). Probiotics are used to improve the health of both animals and humans. In addition, they play an important role in the treatment of diarrhea, lactose intolerance, inflammatory bowel disease, cancer prevention, reduction of serum cholesterol and enhancement of the immune response. Furthermore, it was reported that probiotics may possess *in vitro* and *in vivo* antioxidant activity because they are able to decrease the risk of accumulation of ROS during food ingestion and could potentially be used to reduce oxidative stress (5-7). Their ability to act as antioxidants can be attributed principally to the presence of some antioxidant enzymes such as the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, glutathione reductase

and glutathione-s-transferase, to the release of different antioxidant compounds acting mainly as free-radical scavengers such as glutathione, to the production of some extracellular polysaccharide biomolecules (8-9), or finally, to the exhibition of metal chelating activity (10).

It is well known that paracetamol hepatotoxicity is characterized by an extensive oxidative stress. Paracetamol is mainly detoxified by glucuronidation and sulphation in the liver before being excreted by the kidneys. When ingested in small amounts, paracetamol is converted to N-acetyl-p-benzoquinoneimine (NAPQI) which is removed by the kidneys after conjugation with glutathione (11). However, at high doses, paracetamol increases the formation of free radicals resulting in the depletion of glutathione (GSH) hepatic reserves, which is the main mechanism of its toxicity (11-12). Taking into account all these previous considerations, the aim of the present study was to assess the *in vitro* antioxidant activity of the probiotic bacterium *S. salivarius* and to evaluate its antioxidant efficiency on reducing oxidative stress induced experimentally by paracetamol in rats.

2. Objective

The main objective of our study is to evaluate the antioxidant activity of the probiotic bacterium *S. salivarius* ssp *thermophiles* St.sa *in vitro*, to evaluate its potential hepatoprotective effect to face oxidative stress induced by a toxic dose of paracetamol in Wistar rats, and to clarify how probiotic pretreatment can moderate the response of liver to paracetamol overdose. In this purpose, we analyzed the enzyme activities relevant in the redox state SOD and CAT and their mRNA expression, through this study we intend to understand some molecular and enzymatic mechanisms triggered in liver to fight oxidative damage induced by paracetamol with and without probiotic pretreatment.

3. Materials and Methods

3.1. Bacterial Strain and Reagents

The strain *S. salivarius* ssp *thermophilus* St.sa, was kindly provided by Dr. Tayeb Idoui from the laboratory of Biotechnology, Environment and Health, University of Jijel, Algeria. The culture medium used was de Man Rogosa Sharpe (MRS) broth and agar. 1,1 Diphenyl-2-picrylhydrazyl (DPPH) and thiobarbituric acid (TBA) were procured from Sigma (USA). All other reagents were of analytical grade.

3.2. Assessment of Antioxidant Activity

Bacterial strain was cultured in MRS broth, incubated at 37 °C for 24 h and centrifuged at 4500 xg for 10 min at 4 °C. The bacterial pellet was washed twice with sterile normal saline and resuspended to obtain a concentration of 10^9 CFU/mL.

3.2.1. DPPH Radical Scavenging Activity

Aliquots of 0.1 mL of freshly prepared DPPH in methanol (0.2mM) were mixed and allowed to react with 0.8 mL of intact cells suspension for 30 min at room temperature. The controls included only sterile saline water and DPPH solution, while the blanks contained methanol and the bacterial suspension. The absorbance was measured at 517 nm (13). The test was performed in triplicate. The radicals scavenging activity was determined as follow:

$$\%\ \text{RSA (Radical Scavenger Activity)} = \left(\frac{\text{Abs}_{\text{Control}} - Abs_{Sample}}{Abs_{Control}}\right) x100$$

Where $\text{Abs}_{\text{control}}$ is the absorbance of the control solution and $\text{Abs}_{\text{sample}}$ is the absorbance in the presence of the probiotic strain in DPPH solution.

3.2.2. Resistance to Hydrogen Peroxide

Samples of 20 ml of PBS with 1 mM H_2O_2 were inoculated with a cell suspension containing 10^9 CFU/mL. The cell suspension was prepared after centrifugation of an overnight culture (3000 xg, 15 min). The pellets were washed twice and resuspended into PBS. The inoculated PBS (1 mM H_2O_2) was incubated at 37 °C. The viability of *S. salivarius* in stress condition was monitored as follow: Aliquots of the culture appropriately diluted were taken over 2, 4, 6 h, and plated onto MRS agar plates for counting the number of cells forming colonies, incubation of plates was performed at 37 °C for 48 h (7).

3.2.3. Inhibition of Lipid Peroxidation

In this experiment, plasma lipids were chosen as substrate. Briefly, 400 µl of plasma, 100 µL of $FeSO_4$ solution (50mM) and 0.2 mL of the bacterial suspension were mixed and incubated at 37 °C in a water bath for 15 h. Then, 375 µL o f trichloroacetic acid (TCA) (4%) and 75µL of butylated hydroxytoluene were added and allowed to react or 5 min in ice bath and centrifuged at 2500 xg for 10 min. Aliquots of 200 µL of TBA (0.67% in NaOH 50 mM) were added to the supernatant, followed by an incubation at 100 °C for 30 min, the mixture was left to cool, then, the inhibition of lipid peroxidation was determined at 532 nm (14). The inhibition rate was calculated as follow:

$$\text{inhibition}(\%) = \frac{1-\text{Acontrol}}{\text{Acontrol}} \times 100$$

In the control, the bacterial suspension was replaced by distilled water.

3.2.4. Hydroxyl Radical Scavenging Effect

In this method, o-phenantroline and $FeSO_4$ were used to generate $OH^{\cdot}$ radicals. The hydroxyl radical was generated in the mixture of 1 mL of 0.75 mM 1,10-phenanthroline, 1 mL of 2.5 mM $FeSO_4$, 1 mL of H_2O_2 (20 mM) and 1 mL sodium phosphate buffer (0.2 M, pH 7.4). Then, to determine the probiotic OH scavenging capacity, 1 mL probiotic solution was added, the mixture was incubated at 37 °C for 30 min. The absorbance of the mixture was measured at 536 nm (15). The scavenging activity was determined by the following equation:

$$\text{The scavenging activity on hydroxyl radical (\%)} = \left(\frac{\text{As} - Ap}{Ab - Ap}\right) x100$$

Where (As) was the absorbance of the mixture in presence of probiotic, (Ap) in its absence and (Ab) was the absorbance of distilled water instead of H_2O_2 and probiotic sample.

3.2.5. Iron Ions Chelating Ability

The reaction mixture contained 0.5 ml of cell pellet (10^9 CFU.mL^{-1}), 0.1 mL of ascorbic acid (1%, v/v), 0.1 mL of $FeSO_4$ (0.4 g.L^{-1}) and 1 mL of 0.2 M NaOH. Incubation was carried in a water bath at 37 °C and 0.2 mL of TCA (10%) was added to the mixture and incubated for 20 min. The mixture was then centrifuged at 4500 xg for 20 min and the obtained supernatant was collected and mixed with 0.5 mL of phenanthroline (1 g/L). After a reaction of 10 min, the absorbance was measured at 510 nm against a blank (7). The assay was carried out in triplicate. The chelating ability on iron ions was calculated as follows:

$$\text{\% chelating ability on iron ions} = \left(\frac{\text{Abs}_{\text{Blank}} - Abs_{Sample}}{Abs_{Blank}}\right) x100$$

3.3. Animals and Experimental Design

Female Wistar rats (180-220g) were obtained from Pasteur Institute of Algiers (Algeria) and maintained in standard conditions. The animals had free access to water and food during the experimental protocol. Animals were divided into 4 groups: 1) Healthy control group rats receiving only normal saline in all days of treatment; 2) Probiotic control group receiving a daily dose of 10^9 CFU of probiotic bacteria for 7 days; 3) Paracetamol induced liver injury group, rats in this group were treated by a single dose of paracetamol managed *per os* (200 mg/Kg/body weight),corresponding to 2/3 the LD50 on female albinos Wistar rat (16),and administered in day 7;4) Probiotic protective group receiving a daily dose of 10^9 CFU of probiotic bacteria for 7 days and a single dose of paracetamol managed *per os* (200 mg/Kg/body weight)administered in the last day.

Blood samples were collected and the serum was separated by centrifugation at 3200 xg for 15min. the separated plasma was used for evaluating the biochemical parameters. Animals were sacrificed 24h after paracetamol administration by chloroform anesthesia. The livers were removed and rinsed by normal saline solution and immediately stored at -20 °C for further analysis.

3.3.1. Hepatic Makers as Index of Liver Damage

The liver function markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were selected as an index of liver damage. The assays were performed with an autoanalyzer (DIAMS 2300+) at the Biochemistry Laboratory of Mohamed Seddik Benyahia Hospital (Jijel-Algeria).

3.3.2. Non-Enzymatic Parameters in Liver

The amount of GSH in liver tissue was measured according to the method of Ellman (1959) based on the use of Ellman reactive 5,5-dithiobis-(2-nitrobenzoic acid or DTNB) (17). Briefly, 1 g of liver tissue was homogenized with 3 volumes of KCl (1.15 M), and then, fifty µL of the homogenate was diluted with 10 mL of phosphate buffer (0.1 M, pH 8). Twenty µl of DTNB (0.01 M) was added to 3 mL of the dilution mixture and incubated for 15 min. Glutathione content was determined at 412nm and the hepatic GSH level was expressed as nmol.g^{-1} tissue. Malondialdehyde (MDA), as an endpoint of lipid peroxidation, was evaluated by detecting absorbance of thiobarbituric acid reactive substances at 532 nm according to the method described by Ohkawa *et al*. (18). 0.5 mL of the homogenate was mixed with 1 mL of thiobarbituric acid (0.67%) and 0.5 mL TCA (20%) and heated for 15 min at 100°C. The reaction was stopped by cooling. Two ml of n-butanol was then added to the mixture and centrifuged. The DO of the supernatant was measured at 530 nm. MDA levels were expressed as MDA nmoL.mg^{-1} protein

3.3.3. Enzymatic Activities in Liver

Samples of 1 g of liver were homogenized with 10 volumes phosphate buffer (50 mM, pH 7.0) using a polytron homogenizer and centrifuged at 3000×g for 20 min to remove cell debris, unbroken cells, nuclei and erythrocytes. The supernatant was collected and used for the estimation of antioxidant enzymes activities.

SOD activity was assayed spectrophotometrically based on the formation of formazan salt by the reaction between reduced nicotinamide adenine dinucleotide and phenazine methosulfate–nitroblue tetrazolium. The superoxide radicals produced *in situ* can reduce nitroblue tetrazolium to form formazan crystals (blue), which are detected at 560 nm. One unit of enzyme was defined as the activity needed to reach a 50% inhibition of formazan formation in 1 min (19).

CAT activity was measured by mixing the samples with 50mM phosphate bufferand 30 mM H_2O_2. The amount of hydrolyzed H_2O_2 was calculated by the molar extinction coefficient of H_2O_2 at 240 nm (0.071 mM^{-1} cm^{-1}).The result was expressed as the µM of H_2O_2 decomposed per min per milligram of protein (20).

3.3.4. Gene Expression Analysis

Total RNA was extracted from liver samples by using TRIzol Reagent. It was then quantified and its purity assessed by spectrophotometer (NanoDrop 2000c). The RNA was then treated with DNase I (Promega) to remove genomic DNA contamination. Complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the SuperScript III reverse transcriptase (Invitrogen) with an oligo-dT18 primer. The expression of the selected genes was analyzed by real-time PCR, which was performed with an ABI PRISM 7500 instrument (Applied Biosystems) using SYBR Green PCR Core Reagents (Applied Biosystems). Reaction mixtures (containing 10 µL of 2×SYBR Green supermix, 5 µL of primers (0.6 µM each) and 5 µL of cDNA(template) were incubated for 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 1 min at 60 °C, and finally 15 s at 95 °C, 1 min at 60 °C and 15 s at 95 °C. The used primers were as follow: SOD, sense 5'-CACTCTAAGAAACATGGCG-3', antisense 5'-CTGAGAGTGAGATCACACG-3'; CAT sense 5'-ATGGCTTTTGACCCAAGCAA-3', antisense 5'-CGGCCCTGAAGCTTTTTGT-3' and ß-Actin sense 5'-CCCATCTATGAGGGTTACGC-3', antisense 5'-TTTAATGTCACGCACGATTTC3-'. For each mRNA, gene expression was corrected by the RNA β-actin subunit content in each sample. The results are expressed with respect to the control group, which was normalized to 1. Data of gene expression are represented as fold decrease or increase obtained by dividing each sample value by the mean control value. Values higher than 1 express an increase while values lower than 1 express a decrease in the indicated gene.

3.4. Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA). Post hoc comparisons have been performed using the *t-test* when ANOVA was significant. The values are expressed as means ± S.E.M for 4 rats in each group. *P-Values* < 0.05 were considered significant. All statistical analyses were carried out using Excel SPC software package. Results were considered to be significantly different when $p < 0.05$ and highly significantly different when $p < 0.001$.

4. Results

4.1. In Vitro Antioxidant Activity

The result indicated that the probiotic strain *S. salivarius* showed a considerable free radical scavenging activity against DPPH since it showed a high activity with a percentage of 89.43%. Concerning the impact of H_2O_2 on the viability of *S. salivarius*, the strain can be considered as tolerant to H_2O_2. After being exposed to 1 mM H_2O_2 for 2 h, the cells exhibited a survival rate of 93%, the rate decreased to reach 77% after 6 h of exposure **(Fig. 1)**. Lipid peroxidation was significantly inhibited in the presence of intact cells with a rate of 57%. Fenton chemistry reaction was used in this study to test the scavenging ability of hydroxyl radical, the strain was found to be effective in scavenging these radicals (47%). For the chelating capacity against iron ions the strain showed moderate ability (33.2%).

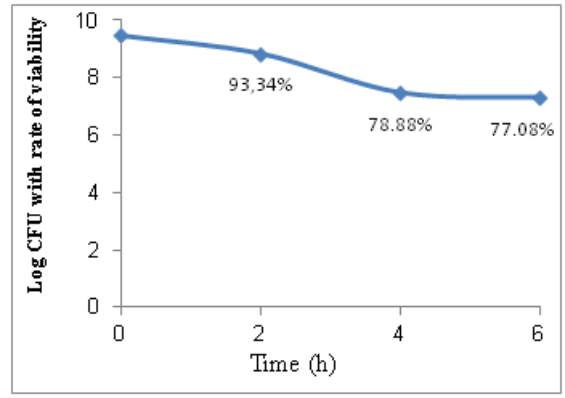

Figure 1. Viability of probiotic S. *salivariu sp* thermophilus to 1 mM hydrogen peroxide, in phosphate buffer at 37 °C.

4.2. Reducing Paracetamol Toxicity (In Vivo)

It is important to mention that, during the experimental protocol no changes were observed on the rats. No rats receiving oral probiotic showed signs of diarrhea, weight loss or difference in food intake. All these remarks indicated the safety of the used probiotic strain. In this study, the association of several biomarkers was chosen for monitoring of paracetamol hepatotoxicity.

4.2.1. Hepatic Marker Enzymes

In order to evaluate liver damage, AST, ALT and ALP enzymes were analyzed. The results illustrated in **Figure.**

2 showed a significant ($p<0.05$) increase in AST, ALT, PAL levels in paracetamol toxicity group which demonstrated the severity of paracetamol toxicity leading to tissue damage. For the group pretreated for one week with *S. salivarius* it can be shown that the level of the enzymes remained unchanged and probiotic can significantly protect the integrity of cell membrane ($p<0.05$) against paracetamol induced alterations of these parameters.

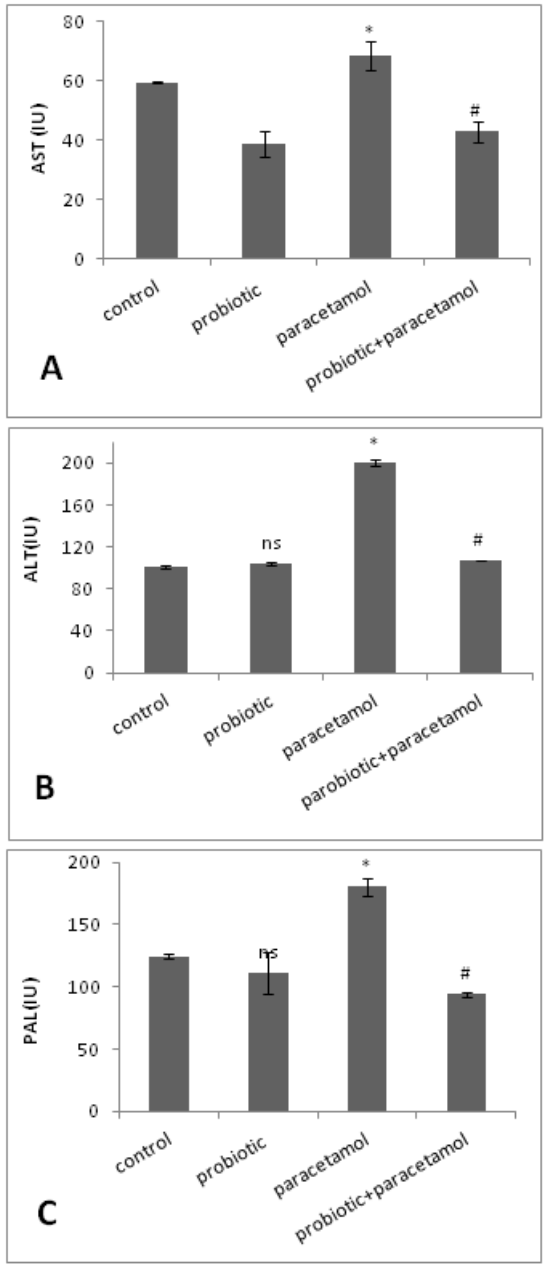

Figure 2. Effects of probiotic on the activities of AST (A); ALT (B) and PAL(C) determined in serum after paracetamol overdose administration with or without probiotic pretreatment. Asterisks (*) denotes significant differences between control rats and paracetamol group ($p < 0.05$). (#) denotes significant differences between paracetamol treated group with probiotic pretreatment relative to rats without probiotic pretreatment. (ns) denotes no significant differences between probiotic relative to control rats

4.2.2. Non-Enzymatic Antioxidants

Evaluation of MDA production in liver of rat from paracetamol treated group, showed a significant elevation of its levels ($p<0.05$) compared with the control. However, a highly significant ($p<0.001$) reduction in probiotic pretreated toxicity was noted. The group treated with probiotics alone showed the same values as those observed in control **(Fig. 3A)**.

On the other hand, we observed a significant depletion in liver glutathione content in the group treated with a toxic dose of paracetamol ($p<0.001$).Thus, the administration of paracetamol toxic dose elevated the level of MDA and decreased GSH levels. Interestingly, the pretreatment for one week with the probiotic strain, significantly restored the liver GSH content ($p<0.001$) **(Fig. 3B)**.

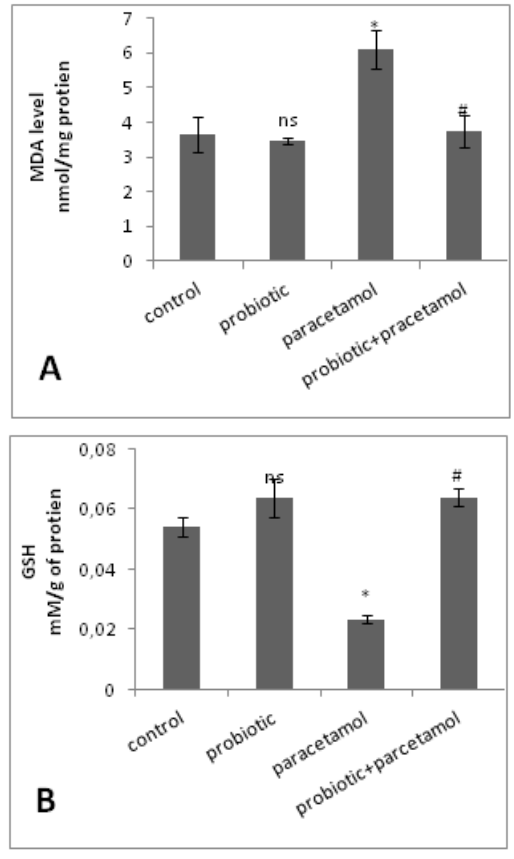

Figure 3. Levels of MDA (A) and GSH (B) in rat liver. Each value is expressed as mean±S.E.M. Asterisks (*) (**) denotes significant and highly significant differences between control rats and paracetamol group ($p < 0.05$). (#) and (##) denotes significant and highly significant differences between paracetamol treated group with probiotic pretreatment relative to rats without probiotic pretreatment. (ns) denotes no significant differences between probiotic relative to control rats

4.2.3. Enzymatic Antioxidants

As shown in **Figure 4A**, the administration of 200 $mg.kg^{-1}$ paracetamol caused a strong significant inhibition ($p<0.001$) of catalase activity. In pretreated rats, probiotic intake can lift the paracetamol inhibitory effect on

enzyme activity where there has been an increase in the activity of this enzyme compared to the group receiving the toxic dose of paracetamol, however, this effect is highly significant ($p<0.001$). In contrast, when rats were treated with *S. salivarius* only, CAT activity decreased significantly ($p<0.001$). **Figure 4B** shows a significant ($p<0.001$) inhibition of SOD activity in rats receiving paracetamol toxic dose. While the preventive treatment with probiotics can lift this inhibition ($p<0.001$). The probiotic alone treated group had no significant changes in SOD activity ($p>0.05$).

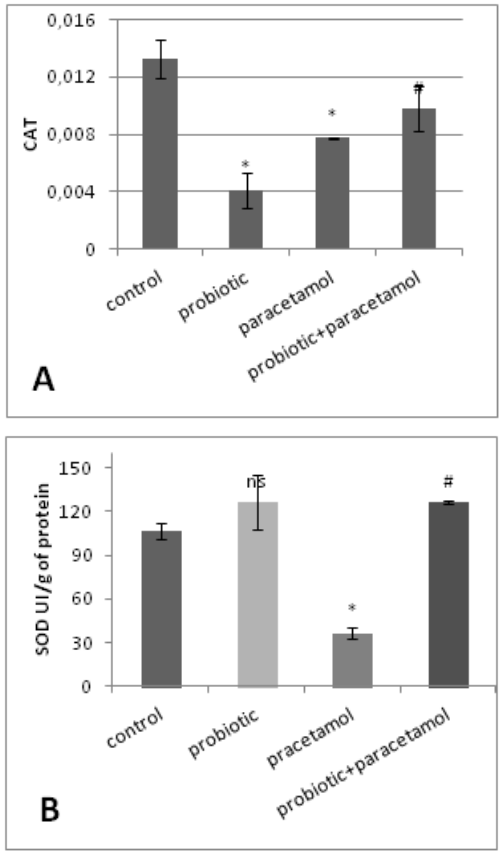

Figure 4. Effects of probiotic on the activities of antioxidant enzymes rat liver. (A) CAT activity; (B) SOD activity. All values are expressed as mean±S.E.M. Asterisks (**) denote highly significant differences between control rats and paracetamol group ($p < 0.05$). (##) denote highly significant differences between paracetamol treated group with probiotic pretreatment relative to rats without probiotic pretreatment. (ns) denotes no significant differences between probiotic relative to control rats.

4.2.4. Gene Expression

In this part, we analyzed whether the level of mRNA expression of antioxidant enzymes SOD and CAT is affected by administration of the paracetamol toxic dose with or without probiotic pretreatment. Our results showed no significant differences in the expression of both SOD and CAT in all groups **(Fig. 5)**. The results indicated unparallel change of antioxidant enzyme activity and its mRNA expression. This molecular finding revealed that probiotic did not attenuate the regulation of antioxidant enzyme gene expression.

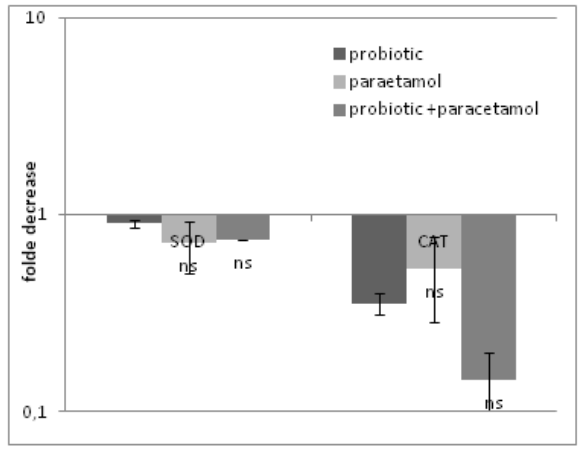

Figure 5. Effects of probiotic on the expression of antioxidant enzymes (SODand CAT) genes determined by real-time PCR in the liver of rats after paracetamol overdose administration. All values are expressed as mean±S.E.M. (ns) denotes no significant differences between control rats and paracetamol group ($p < 0.05$) and between paracetamol treated group with probiotic pretreatment relative to rats without probiotic pretreatment. SOD: superoxide dismutase CAT: catalase.

5. Discussion

Paracetamol overdose causes various pathologies in the liver, most of them are related to oxidative stress, and so, it would be interesting to prevent or to find a way to reduce its side effects.

The use of probiotics may represent an effective strategy to prevent deficiencies of antioxidants, thus new probiotic strains with important antioxidative activity are needed. It is also important to note that the ability to prevent or scavenge ROS is the most promising therapeutic target against paracetamol toxicity. At the center of this focus, our study was undertaken to characterize the antioxidant efficiency of a probiotic *S. salivarius*and to reveal its protective effect against paracetamol hepatotoxicity; although the exact mechanism of such effect is not clear. For this, in the last part of this paper, the expression level of some antioxidant enzyme genes will be considered, in an attempt to clarify a possible mechanism by which probiotic protects against paracetamol induced oxidative stress in liver**.**

Oxidation process involves a cascade of reaction steps. Therefore, the antioxidant activities can be exhibited through multiple reaction mechanisms. Thus, different assays must be performed to provide comprehensive information about the total antioxidant capacity of the tested compound. To characterize the antioxidant efficiency of *S. salivarius,* different assay methods were used.

The highest DPPH scavenging activity of probiotics was also reported; *Probionebacterium freudenreichii*

CFE and *Lactobacillus reuteri* showed high antioxidant activity with percentages of 97.75% and 96.74%, respectively (8). The observed effect may not be attributed to the antioxidant enzymes released from the bacteria but rather to the presence of other molecules released from the bacterial strain (8-9).

Hydrogen peroxide is not a free radical but it is an intermediate during endogenous oxidative metabolism;it, therefore mediates radical oxygen formation (21). The tolerance exhibited by the bacterial strain is not unexpected, because hydrogen peroxide is one of the antimicrobial compounds produced by some lactic acid bacteria (15). Our findings are similar to the results obtained by Lee et al. (22) who found that *Lb. casei* KCTC 3260 remained viable for 8 h in presence of 1 mM H_2O_2.

Lipid peroxidation arises from a series of reactions between free radicals and lipids. The product of lipid peroxidation includes MDA which is considered as an important feature of cellular injury, due to its ability to damage proteins, nucleic acids, and other biological macromolecules resulting in numerous pathological events (23). The inhibitory effect of the strain *S. Salivarius* against lipid peroxidation could be related to its ability to scavenge free radicals and/or to chelate metal ions. Ou *et al*. (14) obtained similar results when they studied the antioxidant activity of the yoghurt bacteria *S. salivarius* ssp *thermophilus* ATCC 19258 and *Lb. delbrueckii* ssp *bulgaricus*.

Lin and Chang (24) found that *Lb. delbrueckii* ssp *bulgaricus* possesses a good hydroxyl radical scavenging ability. Hydroxyl radical is the main factor to cause oxidative damage *in vivo* due to its strong reactivity and oxidizing capacity on almost every type of molecules in living cells (5-25). Elimination of hydroxyl radical plays a critical role in reducing oxidative damage and it is used to predict the scavenging capability of antioxidants in biological systems (21-22).

The chelating capacity against iron ions of our strain is lower than that reported with *Lb. casei* 01 which was able to chelate 72.06% of iron ions (5). Chelating agents could fight toward oxidative stress due to their ability to mask the effect of metal ions (26). Iron ions are highly active metals involved in oxidative processes and participates in hydroxyl radical formation through Fenton reaction (5, 26).

The level of aminotransferase activity is considered as a serum biochemical marker of hepatic injury**.** The increase in AST, ALT, PAL levels in paracetamol toxicity group demonstrated the severity of paracetamol toxicity leading to tissue damage. These enzymes are released in blood stream from the cytosol when cell membrane of hepatocytes is damaged (27). Results obtained after pretreatment with *S. salivarius* illustrated the protective role of this strain against paracetamol hepatotoxicity associated with a non toxic effect of the used strain.

The increase of MDA production in liver of rat treated with a toxic dose of paracetamol indicated that paracetamol has induced oxidative stress. However, reduction of MDA level in probiotic pretreated toxicity may be due to the powerful antioxidant activity and free radical scavenger effect of the probiotic strain. The results are in agreement with other findings indicating that probiotic supplement recovered antioxidant capacity and decreased lipid peroxidation (13, 28). We can suggest that the strain *S. salivarius* had the ability to attenuate oxidative stress by decreasing the lipid peroxidation level.

The depletion in liver glutathione content suggests a detoxification function of the glutathione system which is well reported (29-30). The elevated levels of MDA and the decreased GSH levels indicate a failure of antioxidant defense machinery due to ROS generation. The beneficial effect of the pretreatment with probiotics may be due to the intake and/or the induction of synthesis of non-enzymatic antioxidants such as GSH by probiotics (9-10). In addition, intake of probiotics could induce the transcription of genes involved in the biosynthesis of GSH in the intestinal mucosa of the host (31).

Inhibition of CAT and SOD activity ratifies the toxic effect of paracetamol, as a result of the excessive production and accumulation of ROS production as well as the increase in the observed MDA (32). Mandal *et al*. (33) reported that paracetamol administered to a toxic dose resulted in decreasing or inhibiting of SOD and CAT activity following the generation of ROS. Indeed, the drug causes increased production of microsomol superoxide and hydrogen peroxide in mice. The protective effect of probiotics *S. salivarius* could be related to the secretion of enzymes such as SOD by bacteria to resist against oxidative stress, as several selected strains have very high levels of SOD activity (33).

It should be demonstrated that paracetamol can cause serious toxic effects associated to oxidative stress by the production of NAPQI, a reactive intermediate responsible for oxidant activity, this metabolite is detoxified by GSH, leading to direct relation between paracetamol toxicity and GSH depletion (29). Furthermore, Oliveira et al. (34) reported that paracetamol exposure may induce deleterious effects including enzyme inactivation. Thus, the significant decrease in the antioxidant enzyme activity may be explained as a consequence of direct inactivation of these enzymes caused by reactive metabolites of paracetamol. Castex *et al*. (35) associated the reduction of antioxidant enzymes activity with decreased oxidative

stress and free radical activities. The body expressed fewer antioxidant enzymes due to the lower level of oxidative stress and free radical. These data allowed us to focus our investigations to clarify a possible mechanism by which paracetamol and probiotic exert their effects, through analyzing the expression of the antioxidant enzyme genes. The analysis of the level of mRNA expression of antioxidant enzymes SOD and CAT showed no significant differences in the expression of both SOD and CAT in all groups and this could be due to the mechanisms by which ROS can regulate the transcription factors of gene expression (36). In addition, the changes in oxidative stress parameters may not always require an over-expression of antioxidant enzymes during paracetamol toxicity (37). And this may be due to the fact that paracetamol toxicity was a direct action of its known reactive metabolite NAPQI, rather than a consequence of gene regulation (38). Similar findings were obtained when the effect of paracetamol toxicity on hepatic mRNA expression of SOD, CAT and GSH-Px was investigated. No change was observed in mRNA expression of SOD and CAT in response to paracetamol toxicity in rabbit's liver (37).

On the other hand, the conflicting results concerning SOD and CAT enzyme activities and their mRNA expression is an interesting observation because both activities decreased significantly in response to paracetamol induced oxidative stress. However, no significant change was observed on their mRNA expression in the same experimental group. These findings are consistent with others researches that showed no significant differences in mRNA levels of SOD and CAT enzymes face of a significant oxidative stress (39). To explain this situation we suggest that reduction in SOD and CAT activities is related to increased utilization of these enzymes in scavenging and neutralizing the free radicals and lipid peroxides (40). The activities of SOD and CAT may be affected by several factors, such as post-transcriptional, translational and post-translational modifications, metal binding, H_2O_2 inhibition and are not only related to gene expression (41-42). In this context, Wu et al. (42) showed that the transcriptional responses of SOD and CAT genes to a stress induced in *Oxya chinensis* by Pb were variable. mRNA levels of icCuZnSOD2, ecCuZnSOD1, ecCuZnSOD2, MnSOD and CAT1 were affected by Pb administration, while the transcription of icCuZnSOD1 and CAT2 had no significant changes. The author suggested that the obtained results implied that different regulation mechanisms modulated these genes expression under lead stress. In this case, differences between enzyme activity and gene expression lead to conclude that transcriptional and post-translational modifications might be related to Pb-induced oxidative stress (42).

Finally, we suggest that probiotic protective effect could be related to the reduction of ROS accumulation through their free radical scavenging ability rather than affecting gene expression. Furthermore, this protective effect could be related to many activities such as secretion of enzymes like SOD, metal-chelating activities, promotion of the production of antioxidant biomolecules such as exopolysaccharides showing an *in vitro* free radical scavenging activities (10, 43).

6. Conclusion

The findings of the present study indicated that *S. salivarius* ssp *thermophilus* St.sa displayed interesting antioxidant capacity. In addition the intake of this strain can help rats to resist against oxidative stress induced by paracetamol overdose in liver. However, more in depth research is needed to confirm the suggestion regarding the role of this strain in reducing paracetamol toxicity.

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