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Subchronic toxicity study in BALBc mice of enterocin AS-48, an antimicrobial peptide produced by *Enterococcus faecalis* UGRA10

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ABSTRACT

Few studies have examined the use of animal models to evaluate the *in-vivo* toxicity of antimicrobial peptides, but such research is essential to their safe use in foods. This study was performed to evaluate any adverse effects of enterocin AS-48, a circular bacteriocin produced by *Enterococcus* strains, when administered to BALB/c mice at concentrations of 50, 100, and 200 mg/kg in the diet for 90 days. Animals dosed with nisin at a dietary concentration of 200 mg/kg served as a reference treated group.

There were no deaths in any of the animal groups, and the AS-48 treatment produced no abnormalities or clinical signs on body weights, food consumption, urinalysis, haematology, or blood biochemistry. Furthermore, there were no significant differences in the weights of liver, spleen, heart, kidneys, and intestines between control mice and those treated with AS-48 or nisin. The histopathological study showed moderate vacuolar degeneration in hepatocytes of some animals fed 100 or 200 mg/kg AS-48 (3/10 and 2/10 respectively). However, this anomaly was lower than in the group treated with nisin (5/10). Conclusively, no toxicologically significant changes were associated in BALB/c mice fed with 50, 100, and 200 mg/kg enterocin AS-48 for 90 days.

1. Introduction

Bacteriocin can be defined as ribosomally synthesized antimicrobial peptides produced by bacteria that are active against more or less related bacteria, either in the same species (narrow spectrum) or across genera (broad spectrum) (Cotter et al., 2005). Many of these bacteriocins are produced by lactic acid bacteria (LAB) and found in numerous foods (Alvarez-Sieiro et al., 2016; Perez et al., 2014). Nisin (E234) is at present the bacteriocin most widely used as food preservative (Juncioni de Arauz et al., 2009). Several LAB bacteriocins have been thoroughly characterized so far and there is broad knowledge about their structure and mode of action but some aspects of these compounds, e.g. toxicity, are still rather unknown. Even when toxicity data exist for a few LAB bacteriocins (Gupta et al., 2008; Hagiwara et al., 2010; Sahoo et al., 2017), most studies have been conducted using cell models or acute *in-vivo* tests. Since its use in food preservation implies that these compounds might be taken on a long-term basis, it seems more adequate to study the toxicity aspects of bacteriocins through chronic and subchronic toxicity studies.

Enterocin AS-48 is a circular bacteriocin produced by *Enterococcus faecalis* strains from both clinical (Gálvez et al., 1986; Tomita et al., 1997) and food sources, including the strain *E. faecalis* UGRA10 isolated from a raw sheep's milk farmhouse cheese (Cebrián et al., 2012). Enterocin AS-48 was shown to have antibacterial activity almost exclusively, being most of the Gram-positive bacteria tested highly sensitive to AS-48. Furthermore, early studies showed that some species of Gram-negative bacteria were also inhibited by enterocin AS-48 (Gálvez et al., 1989b). On the contrary this antimicrobial was not active against most of eukaryotic cells such as yeasts and molds, amoeba, or red blood cells (Gálvez et al., 1989b). However, the strong activity of AS-48 against the flagellate protozoa, *Trypanosoma* and *Leishmania,* has been recently referred (Abengózar et al., 2017; Martínez-García et al., 2018). This selective activity is probably due to the strong negative charge of the membrane of both parasites, which is essential for the initial interaction with the cell membrane, the AS-48 target. Several biotechnological applications are being developed currently for this peptide. The most extensively investigated is its use as food biopreservative, since currently, increasing consumer's awareness of the

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potential health risks associated with chemical additives has led us to investigate the possibility of using natural products such bacteriocins produced by LAB or even LAB strains producer of bacteriocins as biopreservatives (Abriouel et al., 2010; López-Cuellar et al., 2016; Montalbán-Lopez et al., 2011; Yang et al., 2014). AS-48 is one of the bacteriocin more investigated as an alternative to conventional preservatives in food. This is due to the great potential of AS-48 to control foodborne pathogen and spoilage bacteria in certain foodstuffs of animal origin (including meats, dairy products, and seafood), as well as many different types of vegetable-based foods (Ananou et al., 2010; Baños et al., 2012; Baños et al., 2016; Muñoz et al., 2007). Nowadays the potential as probiotic of *E. faecalis* UGRA10 is also under study. Preliminary studies on its functional, safety and gut-colonization properties carried out *in vitro* as well as the interference with the adherence of *Listeria monocytogenes* to Caco cells suggest that UGRA10 is a good probiotic to be used in human and/or animal (Cebrián et al., 2012). Although numerous studies have dealt with the structure, mode of action, and the effectiveness of this peptide (Gálvez et al., 1989a, 1989c, 1991; González et al., 2000; Sánchez-Barrena et al., 2003), there is still not enough research related to this enterocin's toxicity, being this aspect essential for achieve its safe use. Thus, the aim of this study was to evaluate the subchronic toxicological potential of AS-48 by providing it in diet to BALB/c mice for 90 days.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Enterococcus faecalis UGRA10 (Cebrián et al., 2012) was used as an AS-48 producer. *E. faecalis* S-47, from our collection, was used as the standard indicator strain for bacteriocin activity assays. Bacterial cultures were maintained at 4 °C on BHI-agar (BHA, Scharlau, Barcelona, Spain) slants.

2.2. Enterocin AS-48 production

AS-48 was produced by culturing the strain *E. faecalis* UGRA10 in a whey-derived substrate, Esprion 300 (ES-300) (DMV Int., Veghel, Netherlands), supplemented with 1% glucose (as described by Ananou et al. (2008). AS-48 was recovered from cultures by cation-exchange chromatography on carboxymethyl Sephadex CM-25 (Abriouel et al., 2003). Eluted fractions were tested for bacteriocin activity against the indicator strain S-47 by the agar well diffusion method (Gálvez et al., 1986). The approximate concentration of AS-48 (in μg/ml) in the preparation was estimated by comparing the diameter of the inhibition halo around the well with a titration curve obtained from purified bacteriocin. Before use, the eluted fractions were dialysed at 4 °C against distilled water through a 2000-Da cut-off membrane to eliminate NaCl, and then sterilized by filtration (0.22 μm, Millipore, Belford, MA, USA). Nisin from *Lactococcus lactis* (N CAS.1414-45-5), used in the present study as a reference material, was supplied by Siveele B.V. (Breda, The Netherlands) with a concentration of 50%, and a nisin potency of 1.000 IU/mg.

2.3. Diet preparation

AS-48 and nisin were incorporated at the required levels into an irradiated Global Rodent 2914 powdered diet (provided by the Animal Experimentation Unit of the University of Granada). Diet preparation was carried out at the Diet Production Unit of the Animal Experimentation Service of the University of Granada. Diets comprised enterocin AS-48 formulated at different concentrations (50, 100, and 200 mg/kg), nisin (200 mg/kg), and a control diet with distilled water. Treatment diets were then sealed in plastic bags and stored at 4 °C. In order to maintain compound stability, fresh diet feed was prepared every three weeks.

2.4. Animals

Ten six-week-old female BALB/c mice were provided by the Animal Experimentation Unit of the University of Granada (Granada, Spain) and were allowed a two-week quarantine and acclimation period. After verifying that the mice were in normal health, they were added to the study at the age of 8 weeks. The mice were housed in transparent polypropylene cages on wood chip bedding in an environment-controlled room. Constant temperature (20–22 °C) and humidity (50–70%) were maintained, and the room was artificially illuminated to provide a cycle of 12 h of light per day. Diets and city tap water were available *ad libitum*. The protocol was carried out according to the guidelines of the Helsinki declaration and was approved by the Ethics Committee of Animal Experiments of the University of Granada (Reference Number 808).

2.5. 90-day feeding study

The procedure followed was similar to that of Hagiwara et al. (2010). 50 mice were allocated to five groups (10 mice per group) using a randomized block design, ensuring that weight distribution and initial mean body weights were similar among the groups.

The animals were fed a diet containing enterocin AS-48 (at doses of 50, 100, and 200 mg/kg), nisin (200 mg/kg), or a control diet (without nisin or AS-48) for 90 days. Clinical signs of abnormality (lethargy, anxiety behaviour, loss of hair, postural disorders), as well as each animal's weight, were monitored. Food and water consumption were recorded.

Urinalysis was conducted for all animals of each group at weeks 4, 8, and 12; a semi-quantitative estimation (URIN-10, SPINREACT, Sant Esteve de Bas, Spain) of pH, density, proteins, glucose, ketones, nitrites, bilirubin, leukocytes, occult blood, and urobilinogen was included. In addition, urinary sediments were analysed by microscopic examination.

After 90 days, the mice were sacrificed by cervical dislocation, and blood was collected by cardiac puncture in EDTA-containing tubes in sterile conditions. In order to assess possible changes in physiological functions due to the oral administration of the bacteriocin, several biochemical and haematological parameters were analysed at the Bioanalysis Unit of the Scientific Instrumentation Centre of the University of Granada. Haematological estimations were carried out using an automatic haematology counter Mythic 22-CT (Orphée, Geneva/Plan-les-Ouates, Switzerland) for the erythrocyte count (RBC), white blood cell count (WBC), haemoglobin (HGB), hematocrit (HCT), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and percentage of white blood cell differential count.

Blood biochemistry was determined with a BS-200 automatic chemistry analyzer (Mindray Medical International Ltd., Shenzhen, China) on the following parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (CRE), glucose (GLU), albumin (ALB), total cholesterol (T-CHO), phospholipid (PL), triglyceride (TG), total protein (TP), sodium (NA), potassium (K), and chlorine (CL).

Furthermore, the following organs were removed and weighed from each mouse: heart, spleen, thymus, kidney, liver, and intestines. A full histopathological examination was performed at the AnaPath laboratories (Granada, Spain) on haematoxylin and eosin-stained tissue sections of the liver, kidney, stomach, and intestines for all the mice, including the control group, the nisin group, and the group of mice fed the highest dose of AS-48.

2.6. Statistical analysis

All results are expressed as the mean ± SD. Differences between means were tested for statistical significance using a one-way analysis of variance (ANOVA) and the Student's t-test. Statistical analyses were performed using the SPSS-PC 14.0 software (SPSS, Chicago, Ill. USA)

with statistical significance set at $P < 0.05$.

3. Results and discussion

In-vivo toxicity assessment of each antimicrobial peptide is an important step forward to consider their use as a food preservative. Few studies have been conducted to evaluate the *in-vivo* toxicity of LAB bacteriocins due to the fact that LAB (and therefore their bacteriocins) are widely found in foods and are regarded as safe. Nisin is by far the most commonly studied bacteriocin from a toxicological point of view, with numerous studies on cellular models and experimental animals (Frazer et al., 1962; Gupta et al., 2008; Hagiwara et al., 2010; Sahoo et al., 2017). The present work aims to analyse the toxicological effects of oral administration of enterocin AS-48 for 90 days.

For this purpose we have established five experimental groups (10 mice in each group): three groups were fed a diet containing enterocin AS-48 at doses of 50, 100, and 200 mg/kg respectively; one group was fed a diet containing nisin (200 mg/kg); and a control group was fed a diet that did not contain any of the antimicrobials. We have selected these doses of AS-48 because they are similar or exceeding to the amounts of bacteriocin that could be ingested when foods are supplemented with AS-48 to prevent their microbial alteration or the growth of pathogens. In this respect it should be noted that effective AS-48 concentrations in foods are very variable depending strongly on the fat and protein content and the complexity of food matrix. Thus, in vegetable foods and beverages effective AS-48 concentrations ranged from 12.5 to 25 μg/ml (e.g. in energy drinks against *Staphylococcus aureus*) to 80 μg/ml (e.g. in vegetable sauces against *S. aureus*) (Abriouel et al., 2010). In meat foods effective concentrations against this pathogen were 40 μg/g in sausages (Ananou et al., 2005) and 60 μg/g combined with sodium pyrophosphate in a model cooked ham (Ananou et al., 2010). Sodium pyrophosphate showed a potent synergistic anti-staphylococcal effect with AS-48 since AS-48 alone failed to control *S. aureus* in this food. From the data presented in Table 1, it is possible to calculate the total daily AS-48 amount intakes and then, the rate μg AS-48/g mice. Thus, mice fed a diet containing 200 mg/kg AS-48 received (according to the daily food intake) an daily amount of bacteriocin (in μg/g of mice) ranging from 24.56 μg/g (at 0 days) to 24.92 μg/g (at 90 days). For mice fed a diet containing 100 mg AS-48/kg, amounts received per mouse body weight were 13.59 and 12.66 μg/g at 0 and 90 days, respectively. Finally, for the diet added with the lowest AS-48 concentration (50 mg/kg), AS-48 amounts per mouse body weight were 6.53 and 6.17 μg/g at 0 and 90 days, respectively. For mice fed diet added with nisin at the unique concentration assayed, 200 mg/kg diet, nisin concentrations at the beginning and final of experiment were 27.55 and 26.43 μg nisin/g of mice, respectively. If we considered the equivalent picture for human with an average woman-man body weight of 70 kg and an average food intake of 1.72 kg per person per day (data published by the Spanish Ministry of Agriculture, Fishing and Food, 2018), the total intake of AS-48 through a diet containing 200 mg/kg will be 344 mg, which represents an AS-48 concentration per body weight of 4.91 μg/g. This concentration is much lower than that reached in mice, all the more so if we take into account that it is almost impossible that in humans all daily-diet foods contain AS-48 and that, as said above, bacteriocin concentrations, alone or combined with other chemical or physical treatment, will be quite lower than 200 μg/g food or even 100 μg/g food.

No deaths were observed in the control, nisin, or AS-48 treated groups during the course of the study. Regarding clinical signs, no signs of abnormality were observed in any of the experimental groups. Furthermore, during the experimental protocol, neither noticeable activity nor behavioural changes were observed in the mice. During the 90-days of feeding, the diets with nisin and AS-48 had no adverse effects on food intake, and there was no difference in food intake between the control and the treated groups (Table 1). Neither significant differences were observed concerning body weight gain among the groups (Table 1).

In agreement with the absence of clinical symptoms in mice, the biochemical and haematological parameters measured in blood samples did not statistically differ between control mice and mice treated with 50, 100, or 200 mg/kg enterocin AS-48 (Tables 2 and 3). No significant changes were observed in the nisin-treated group either. Urinalysis also showed no differences between the control group and mice treated with enterocin AS-48 or nisin (results not shown).

Concerning tissue weights, there were no significant differences in the weights of the liver, spleen, thymus, heart, and kidney between control and mice treated with enterocin AS-48 or nisin (Table 4). Furthermore, the weight/length ratios of the small and large intestines did not statistically differ among the experimental groups. In addition to weight, the colouring and appearance of the analysed tissues was normal and no significant differences were observed among the groups.

The pathological anatomy study showed no significant abnormalities in the organs heart, spleen, thymus, kidneys, and small and large intestines for control animals or for those treated with AS-48 and nisin. The histopathological study of the small and large intestines fed the highest dose of AS-48 (200 mg/kg) can be seen in Picture 1. It should be mentioned that the most common finding was vacuolar degeneration in the hepatocytes (Picture 2). In this case, the presence was AS-48 dose-dependent, that is, mice treated with higher bacteriocin concentrations showed a higher presence of vacuoles. No animal with a diet containing 50 mg/kg of AS-48 showed vacuolar degeneration (Picture 2C). Two mice of the group fed with 100 mg/kg of AS-48 diet and 3 mice of the group fed with 200 mg/kg of AS-48 diet showed moderate or mild vacuolar degeneration (Picture 2D). Vacuolar degeneration is a common finding in mice, especially in females, which physiologically appears after food intake and it is generally a reversible change (Choi et al., 2017; Jall et al., 2017; Yip and Burt, 2006). In this case, in animals treated with nisin, vacuolar degeneration was more evident than in the control group and the group fed with AS-48 since this alteration was found at mild to moderate levels in 5 out of 10 animals analysed (Picture 2B). That is, the mice that received the diet with enterocin AS-48 at concentrations of 100 and 200 mg/kg showed more degeneration compared with the control animals, but had less degeneration than the group treated with nisin. However, the oral sub-chronic administration caused no significant changes in blood biochemistry, and specifically, levels of AST and ALT (transaminases), which are used as biomarkers predicting possible hepatic toxicity, did not increase in treated mice and were similar to levels in control mice. In summary, since these histological findings does not correlate to changes in biochemical parameters that indicate hepatic function alterations, it is probable that

Table 1
Body weight and food intake of control and nisin and enterocin AS-48 treated mice.

	Control	AS-48 (mg/kg)			Nisin (mg/kg)
		50	100	200	200
Initial weight (g) time 0 d	21.45 ± 1.04	21.37 ± 1.09	21.34 ± 1.31	21.52 ± 1.42	21.34 ± 1.20
Final weight (g) time 90 d	22.24 ± 1.57	22.69 ± 1.15	22.91 ± 1.25	22.07 ± 1.66	22.25 ± 0.97
Food intake (g/mice/day)	2.95 ± 0.42	2.80 ± 0.62	2.90 ± 0.51	2.75 ± 0.32	2.94 ± 0.24

Values are means ± S.D. for groups of 10 mice.

Table 2
Tissue weights and intestine weight/length ratio of control and mice fed a diet containing nisin or AS-48 for 90 days.

	Control	AS-48 (mg/kg)			Nisin (mg/kg
		50	100	200	200
Liver (mg)	995.5 ± 55.2	982.2 ± 25.1	988.3 ± 70.2	981.7 ± 94.5	945.1 ± 112.2
Spleen (mg)	92.5 ± 14.5	82.3 ± 11.2	95.1 ± 10.5	91.2 ± 7.5	98.5 ± 15.1
Kidney (mg)	129.2 ± 9.2	140.5 ± 21.4	119.2 ± 19.3	153.2 ± 13.4	175.2 ± 32.3
Small intestine (g/cm)	25.7 ± 5.2	22.7 ± 6.2	28.7 ± 7.1.2	25.7 ± 6.3	27.7 ± 3.3
Large intestine (g/cm)	26.9 ± 6.7	28.5 ± 7.5	30.5 ± 10.5	28.5 ± 8.5	29.5 ± 5.1

Values are means ± S.D. for groups of 10 mice.

Table 3
Haematology data of control and mice fed a diet containing nisin and AS-48 for 90 days.

	Control	AS-48 (mg/kg)			Nisin (mg/kg)
		50	100	200	200
WBC (10^3/μL)	0.64 ± 0.21	0.81 ± 0.28	0.80 ± 0.51	1.03 ± 0.23	0.88 ± 0.25
RBC (10^6/μL)	5.27 ± 1.32	5.76 ± 1.32	5.39 ± 1.58	5.86 ± 1.24	6.18 ± 1.29
HGB (g/dL)	7.376 ± 1.10	7.05 ± 1.92	7.52 ± 1.94	8.57 ± 1.08	8.9 ± 1.46
HCT (hematocrit)	20.93 ± 5.23	24.53 ± 12.5	24.32 ± 6.14	24.81 ± 5.35	24.15 ± 6.50
MCV (fL)	42.54 ± 0.51	42.84 ± 1.15	42.44 ± 1.08	42.36 ± 0.52	41.81 ± 1.19
MCH (pg)	16.31 ± 0.32	11.95 ± 0.95	15.06 ± 1.53	17.7 ± 0.93	18.09 ± 0.61
MCHC (g/dL)	38.5 ± 0.58	29.04 ± 1.71	24.6 ± 2.32	37.09 ± 2.24	40.8 ± 1.91
PLT (10^3/μL)	610.32 ± 52.32	643.71 ± 52.51	682.5 ± 76.52	680.5 ± 62.69	625.66 ± 71.5
Leukocyte formula					
LYM%	93.50 ± 3.42	93.50 ± 2.52	92.14 ± 2.70	93.27 ± 1.73	94.20 ± 4.2
MON%	0.35 ± 0.12	0.35 ± 0.05	0.41 ± 0.19	0.36 ± 0.25	0.23 ± 0.5
NEU%	5.08 ± 2.29	4.36 ± 2.24	4.18 ± 2.28	4.55 ± 1.22	3.42 ± 2.2
EOS%	1.71 ± 0.52	2.19 ± 1.63	2.81 ± 1.25	2.16 ± 1.30	2.21 ± 0.4
BAS%	0.36 ± 0.51	0.29 ± 0.53	0.63 ± 0.62	0.04 ± 0.11	0.25 ± 0.61

White blood cell count (WBC), erythrocyte count (RBC), haemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT), lymphocytes (LYM), monocytes (MON), neutrophils (NEU), eosinophils (EOS), basophils (BAS).
Values are means ± S.D. for groups of 10 mice.

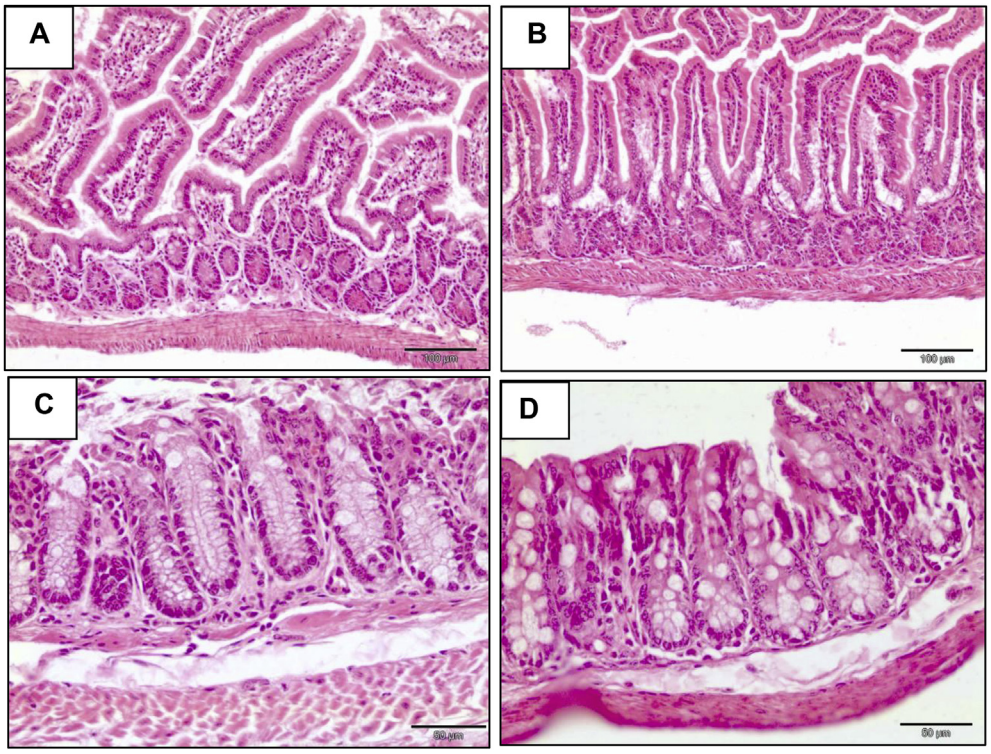
these signs are not directly related to the treatments (Da Silva et al., 2014; Gautam and Goel, 2014; Lu et al., 2014).

Most authors have found no evidence of significant changes in the serum biochemical markers, histopathological analysis or visual observation of organs of mice or rats fed nisin (Gupta et al., 2008; Hagiwara et al., 2010; Sahoo et al., 2017). Nevertheless, other data reported in bibliography on *in-vivo* nisin toxicity are rather contradictory. Almeida Vaucher et al. (2011) referred signs of toxicity in mice treated orally by gavage with 0.825 mg/kg/day of nisin for 21 days, with notable histological changes in the spleen, skin and liver. Also, an increase in the spleen gross organ size and especially in its weight was observed. This find, together with the presence of megakaryocytes in the histological preparations, isuggests a possible inflammatory process. Livers of mice treated with nisin showed an important hepatic degeneration and the presence of neutrophils. In addition, aspartate aminotransferase levels in serum were significantly increased in the mice within the nisin-treated group after 21 days. These results suggest a potential hepatotoxic effect of nisin. In the same study, the authors referred the presence of megakaryocytes in the spleen of mice fed the antimicrobial peptide P34 produced by *Bacillus* sp. strain P34. Other studies showed low *in-vivo* toxicity in bacteriocins such as bovicin HC5, with lower weight gain and slight changes in the small intestine of mice

Table 4
Blood biochemistry data of control and mice fed a diet containing nisin or AS-48 for 90 days.

	Control	AS-48 (mg/kg)			Nisin (mg/kg)
		50	100	200	200
ALT (U/L)	51.47 ± 5.25	49.41 ± 18.60	57.08 ± 9.55	50.42 ± 6.83	54.89 ± 23.12
AST (U/L)	257.633 ± 75.65	166.82 ± 26.05	252.08 ± 35.89	258.84 ± 25.45	267.31 ± 88.14
ALB (g/dL)	4.66 ± 0.68	4.85 ± 0.58	4.12 ± 0.24	4.85 ± 0.35	4.03 ± 0.79
GLU (mg/dL)	50.35 ± 7.41	47.69 ± 11.04	56.81 ± 12.17	55.34 ± 13.03	58.51 ± 13.85
CRE (mg/dL)	0.26 ± 0.04	0.28 ± 0.05	0.26 ± 0.02	0.3 ± 0.09	0.31 ± 0.03
T-CHO (mg/dL)	84.32 ± 9.53	84.34 ± 6.90	83.29 ± 13.84	85.93 ± 5.43	91.26 ± 7.29
TG (mg/dL)	139.12 ± 24.66	134.84 ± 29.33	133.63 ± 24.24	142.31 ± 2.94	142.31 ± 22.53
TP (g/dL)	6.26 ± 0.51	6.96 ± 0.75	6.76 ± 0.87	6.94 ± 0.74	6.94 ± 0.90
PL (mg/dL)	134.82 ± 12.85	136.25 ± 7.0	130.49 ± 15.22	129.47 ± 6.47	149.67 ± 13.49
NA (mg/dL)	414.32 ± 121.05	514.26 ± 108.02	513.18 ± 94.95	521.62 ± 82.05	526.21 ± 119.61
CL (mg/dL)	310.73 ± 36.68	355.01 ± 27.18	325.09 ± 17.92	323.47 ± 51.74	326.64 ± 42.71

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), creatinine (CRE), glucose (GLU), total cholesterol (T-CHO), phospholipid (PL), triglyceride (TG), total protein (TP), sodium (NA), potassium (K), and chlorine (CL).
Values are means ± S.D. for groups of 10 mice.

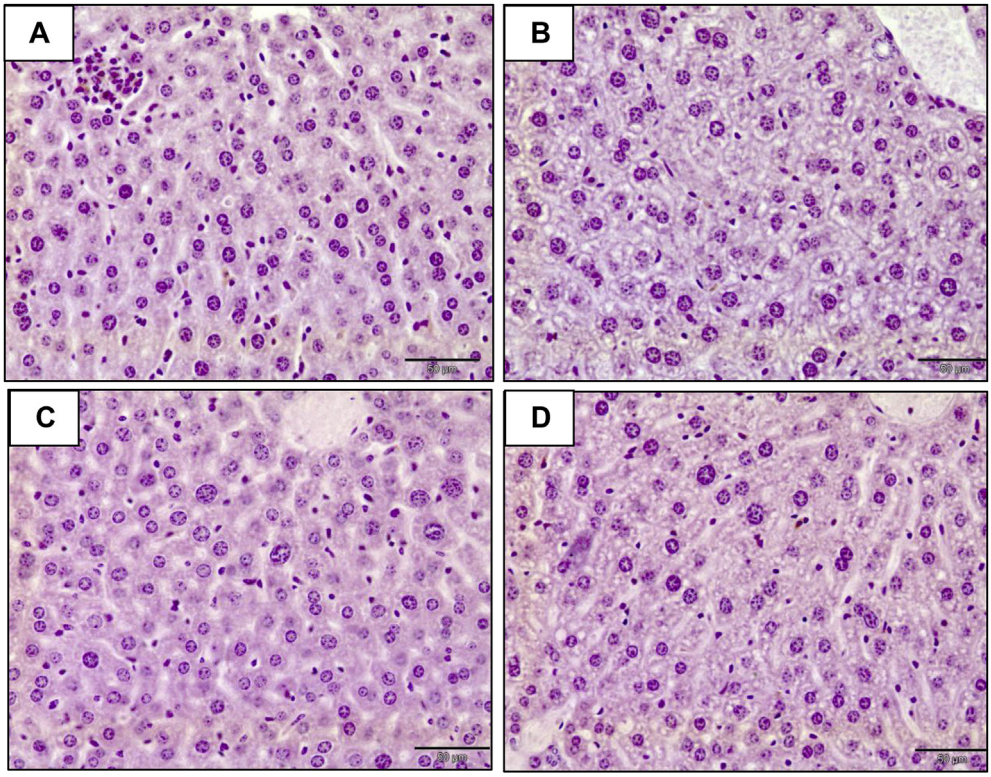


Picture 1. Histopathological examination of haematoxylin and eosin-stained sections of small and large intestines of mice fed AS-48 (200 mg/kg) for 90 days. A) Small intestine of control mice; B) small intestine of AS-48 fed mice; C) large intestine of control mice; D) large intestine of AS-48 fed mice. A, B ——: 100 μm; C, D ——: 50 μm.

(Paiva et al., 2013).

In conclusion, our results indicate that bacteriocin AS-48, administered at dietary levels of up to 200 mg/kg for 90 days in BALB/c mice, does not cause adverse effects on any of the aspects evaluated: food and water intakes, body weight, urine and blood biochemical/haematological parameters. Furthermore, the study carried out on heart, spleen, thymus, kidneys, and small and large intestines did not reveal any alterations in such organs.

Small degenerative changes were observed in the liver of mice fed 100 and 200 mg/kg AS-48, but no other abnormal signs were found concerning the liver function, including hematological and serum biochemistry tests.



Picture 2. Histopathological examination of haematoxylin and eosin-stained sections of liver of mice fed AS-48 for 90 days. A) Liver of control mice; B) liver of mice fed nisin (200 mg/kg); C) liver of mice fed AS-48 (50 mg/kg); D) liver of mice fed AS-48 (200 mg/kg). ——: 50 μm.

According to these results, bacteriocin AS-48 could be deemed, at first, suitable for use as a food preservative. Nevertheless, further study should be conducted by extending the timing to delve further into this issue.

Conflicts of interest

No known conflicts of interest.

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Transparency document

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