

Antiulcerogenic activity of peptide concentrates obtained from hydrolysis of whey proteins by proteases from *Cynara cardunculus*

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A B S T R A C T

Peptide concentrates generated by hydrolysis of whey with aqueous extracts of flowers of *Cynara cardunculus* were studied for possible protection of the stomach mucosa against ulcerative lesions caused by oral administration of absolute ethanol. Both the whole peptide fraction obtained via hydrolysis of whey protein concentrate (peptide concentrate, PepC) and its fraction below 3 kDa (PepCF) were able to reduce gastric injuries to significant levels ($p < 0.05$). Single-dose experiments, using 100 mg kg^{-1} body weight (bw) of either PepCF or PepC, led to 68.5% and 37.4% protection, respectively – which compare well with 93.4% protection by 200 mg kg^{-1} bw carbenoxolone (a positive control). No dose-response correlation could be demonstrated. Gastric cytoprotection by PepCF appears to depend on sulphhydryl-containing moieties, whereas PepC likely protects the gastric mucosa via the prostaglandin cycle and production of nitric oxide.

1. Introduction

Milk has been reported to contain several functional ingredients with favourable effects upon health (Gill, Rutherford, & Cross, 2000; Korhonen, 2009); and whey proteins in the form of concentrates (WPC) and isolates (WPI), or their hydrolysates (WPH), have been reported to possess functional properties well beyond their nutritional value (Clare & Swaisgood, 2000; FitzGerald, Murray, & Walsh, 2004; Gauthier, Pouliot, & Saint-Sauveur, 2006; Lee, Skurk, Hennig, & Hauner, 2007; Meisel, 2005; Meisel & FitzGerald, 2003; Virtanen, Pihlanto, Akkanen, & Korhonen, 2007; Yamamoto, Ejiri, & Mizuno, 2003).

Classical approaches to produce bioactive peptides encompass enzymatic hydrolysis of protein feedstocks; however, whey proteins are not easily broken down by most proteases, e.g., pepsin and trypsin (Schmidt, Meijer, Slangen, & van Beresteijn, 1995). Since enzymatic hydrolysis has primarily used aspartic proteases of animal origin, characterization of similar enzymes of plant origin is in order. Several wild plants produce proteolytic enzymes that are easily extracted with water. This is the case of the dried flowers of *Cynara cardunculus* – a thistle related to the globe artichoke that grows

spontaneously in dry, stony areas in Southern Portugal (Roseiro, 1991), and possesses strong aspartic proteases (cardosins). Such enzymes have acidic pH optima (4.0–6.0), and possess an unusually wide specificity towards cleavage of peptide bonds near hydrophobic amino acid residues (Barros & Malcata, 2006; Veríssimo et al., 1996). Previously, aqueous extracts of *C. cardunculus* flowers were found to exhibit significant proteolytic activity when whole whey or whey protein concentrates were tested (Barros et al., 2003; Barros, Ferreira, Silva, & Malcata, 2001; Barros & Malcata, 2002, 2004; Lamas, Barros, Balcão, & Malcata, 2000).

The gastric mucosa is crucial in protecting the human tissues from low pH and strong digestive proteases; hence, pathological processes may easily occur upon chronic exposure to damaging agents (Melo, Castro, Lanna, Guimaraes, & Sobrinho, 1993). Available evidence indicates that peptic ulcerogenesis (gastric or duodenal) results from an imbalance between protective substances, viz. mucus, bicarbonate, prostaglandins, PG, and such sulphhydryl, SH, compounds as proteins and glutathione, GSH, on the one hand, and blood flux to the mucosa cells, *Helicobacter pylori* infection, and or exposure to damaging chemical agents, on the other (Abdel-Salam, Czimmer, Debreceni, Szolesanyi, & Mózsik, 2001; Allen, Flemstrom, Garner, & Kivilaakso, 1993; Barocelli et al., 1997; Konturek, 1990; Robert, 1979; Szabo, Nagy, & Pevebani, 1992). Nitric oxide, NO, appears to be important against gastric mucosal injury induced by ethanol or endotoxins (Nishida, Ohta, & Ishiguro, 1997; Qiu, Pfeiffer,

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& Cho, 1996; Zhang, Zhang, Chen, & Wang, 2000), and when coupled with L-arginine, it constitutes an alternative therapeutic approach. In the specific case of infection by *H. pylori*, antibiotics are almost exclusively used – yet compounds with gastroprotective effects would represent a promising alternative approach for treatment of peptic gastric ulcers (Barocelli et al., 1997).

Whey proteins are quickly absorbed after entering the gastrointestinal tract (Boirie et al., 1997), but their hydrolysates are absorbed even faster (Grimble & Grimble, 1998). Furthermore, if such hydrolysates contain mainly oligopeptides, they will be less susceptible to upstream denaturation/precipitation caused by pasteurization or sterilization (Adibi, & Morse, 1971; Clemente, 2000). A few studies (Matsumoto, Shimokawa, Ushida, Toida, & Hayasawa, 2001; Mezzaroba et al., 2006; Pacheco et al., 2006; Rosaneli, Bighetti, Antônio, Carvalho, & Sgarbieri, 2002, 2004) have indicated that acute or consecutive oral administration of WPC, WPH, whey protein hydrolysate fraction (WPHF) or α -lactalbumin, α -La, can protect the gastric mucosa from damages induced by ethanol, indomethacin or other ulcerogenic agents, via SH compounds (chiefly GSH) that activate endogenous PG synthesis.

Therefore, the purpose of this research was to elucidate the capacity of a less conventional peptide concentrate, obtained from whey incubated with aqueous extracts of *C. cardunculus* flowers, to protect the stomach mucosa of rats against ulcerative lesions. All tests were conducted following international regulations pertaining to animal experimentation, and under supervision of a bioethical committee. Mechanisms for the protection observed were hypothesized and experimentally tested.

2. Materials and methods

2.1. Animals and feeding

Male Wistar rats (216 in total), weighing 250–300 g each, were purchased from the Experimental Animal Centre (CEMIB) of Campinas University (São Paulo, Brazil). All animals were kept under controlled environmental conditions (20 °C and 12 h light/dark cycle), and supplied with a normal standard laboratory diet (Nuvilab, from Nuvital Nutrients, Curitiba, Brazil) and tap water ad libitum, for at least 1 week prior to the experimental treatment.

2.2. Peptide concentrates

The WPC was obtained from bovine sweet whey, released during regular cheesemaking using bovine milk. Microfiltration was used for sterilization, followed by ultrafiltration/diafiltration for concentration up to 63% protein (34% lactose and 2% lipids), on a total solids mass basis. A hydrolyzate was then produced via incubation of WPC with a commercial aqueous (crude) extract from dried flowers of *C. cardunculus* (Formulab, Maia, Portugal). The enzyme/substrate, E/S, ratio was set at 1.6% (v/v), using 15 g protein L⁻¹ enzyme solution and 40 g protein L⁻¹ WPC solution. This mixture was incubated at pH 5.2 and 55 °C, which have been claimed by Barros and Malcata (2002) to be the optimum processing conditions; hydrolysis was halted after 7 h by heating at 95 °C for 15 min. This step was followed by ultrafiltration, aimed at separating peptides from non-hydrolyzed proteins.

The permeate yielded a peptide concentrate, PepC, that was freeze-dried and kept at room temperature until use; its final composition was 40% (w/w) protein, on a dry basis. A portion of the PepC solution (at 100 g protein L⁻¹) was ultrafiltered through a hydrophilic 3 kDa cut-off membrane (Centriprep, from Amicon, Beverly, MA, USA); the permeate, PepCF, was freeze-dried and

stored at 4 °C prior to use. To prepare the peptide solutions, the protein content of either PepC or PepCF was estimated via the bicinchoninic acid assay (Pierce, Rockford, IL, USA), using bovine serum albumin as the standard.

2.3. Induction of ulcerative lesions

To induce lesions in the gastric mucosa, absolute ethanol was used as an ulcerogenic agent, as described by Robert (1979). The complete experimental protocol was previously approved by the Ethics Committee for Animal Research of Campinas University, in agreement with the ethical principles in animal research adopted by the Brazilian College of Animal Experimentation, COBEA (protocol No. 2118-1).

The animals were divided into groups of 6 rats, one for each treatment considered. Both a negative control, i.e., 0.9% (w/v) sodium chloride solution, and a positive control, i.e., 200 mg kg⁻¹ body weight (bw) carbenoxolone (C4790, Sigma, St. Louis, MO, USA) were considered. A better discrimination was thus expected between percent decrease of the ulcerative lesions index (ULI) associated with the various situations to be tested; note that carbenoxolone, an antiulcerogenic drug, possesses a strong specific capacity to inhibit mucosa lesions caused by ethanol. All rats received gastric intubations of ethanol. Two distinct models, viz., acute and consecutive, were pursued for oral administration of PepC and PepCF.

For the consecutive daily doses, rats were treated over three days (with free access to water). On the first and second days of treatment, animals were fasted for 6 h before administration of the peptide concentrate; chow was then provided for 18 h on the first day, and 8 h on the second day, followed by fasting for 16 h until the third treatment. By 30 min after completion of the last treatment, all rats received absolute ethanol orally (ulcerogenic agent), and 1 h later the animals were sacrificed by cervical displacement. Their stomachs were then removed and opened along the greater curvature, and washed with saline solution to permit unambiguous assessment and evaluation of ulcerative lesions. Tests using a single dose were also performed, which basically omitted the first two days of the experiment pertaining to the consecutive daily doses.

The two concentrates (PepC and PepCF) were dissolved to 10 mL kg⁻¹ bw saline solution, and were administered by gastric intubation at 30, 100, 300 and 350 mg kg⁻¹ bw for the single-dose treatment, and at 100, 200 and 350 mg kg⁻¹ bw for the triple-dose treatment (Table 1).

2.4. Analysis of ulcerative lesions

The ULI score for each animal was calculated by summing the values (Gamberini, Skorupa, Souccar, & Lapa, 1991) as follows: petechial points (<10), 2 points; petechial points (>10), 3 points; ulcers (up to 1 mm), 2 points per ulcer; ulcers (>1 mm), 3 points per ulcer; perforated ulcers, 4 points per ulcer; haemorrhage, 1 point; mucosa oedema, 1 point; loss of normal morphology, 1 point; and discoloration of mucosa, 1 point. The percent ulcerative lesion inhibition (ULI %) was calculated by comparing the lesions in each sample with those in the negative control, according to:

$$\text{ULI \%} = \frac{[(\text{average negative control} - \text{average test sample}) / \text{average negative control}] \times 100}{}$$

Attempts to determine the dose required to lower ULI by 50% (DE₅₀) were based on the single-dose protocol, and increasing doses (30, 100, 300 and 350 mg kg⁻¹ bw) of PepC and PepCF were orally administered to the rats after fasting for 16 h. DE₅₀ was

Table 1

Dose-effects, in terms of the ulcerative lesions index, ULI (mean \pm standard deviation, SD), of treatments with peptide concentrate PepC and its fraction below 3 kDa PepCF, regarding protection in rats against stomach ulcers induced by oral administration of ethanol (with saline solution as negative control, and carbenoxolone as positive control).

Compound administered by intragastric intubation	Number of replicates	Dose (kg ⁻¹ bw)	ULI ^a	ULI fractional change (%)
<i>Single-dose treatment</i>				
PepC	5	30 mg	53.6 \pm 11.3**	43.3
	5	100 mg	59.2 \pm 18.3**	37.4
	6	300 mg	45.2 \pm 26.0*	52.3
PepCF	4	350 mg	55.8 \pm 14.0**	41.1
	6	30 mg	59.3 \pm 30.9**	37.3
	5	100 mg	29.8 \pm 18.5*	68.5
	6	300 mg	64.7 \pm 14.7***	31.6
	4	350 mg	30.5 \pm 4.2*	67.8
Carbenoxolone	5	200 mg	6.2 \pm 2.3*	93.4
Saline solution	5	10 mL	94.4 \pm 11.1	–
<i>Triple-dose treatment</i>				
PepC	4	100 mg	58.8 \pm 8.4*	45.8
	4	200 mg	71.5 \pm 7.3*	34.0
	4	350 mg	92.3 \pm 14.0***	14.8
PepCF	4	100 mg	76.3 \pm 16.3*	29.6
	4	200 mg	50.0 \pm 13.6*	53.8
	4	350 mg	59.5 \pm 10.4*	45.1
Carbenoxolone	5	200 mg	7.2 \pm 2.9*	93.4
Saline solution	5	10 mL	108.3 \pm 3.9	–

^a Asterisks indicate statistical differences: * $p < 0.001$; ** $p < 0.01$; *** $p < 0.05$ (Duncan's test based on saline solution).

calculated by interpolation, after linear regression to the experimental data.

2.5. Protection from ulcerative lesions by sulphhydryl compounds

The contribution of SH compounds to protect rats against stomach ulcerative lesions was estimated using a single-dose trial – with one control group and two experimental groups. All animals received a subcutaneous injection of 10 mg kg⁻¹ bw N-ethylmaleimide, NEM (NEM-E1271, Sigma), as described by Szabo, Trier, and Frankel (1981). After 30 min, the rats in the control group received 10 mL kg⁻¹ bw saline solution orally, whereas those in the other two groups received 100 mg kg⁻¹ bw of either PepC or PepCF dissolved in a saline solution. After another 30 min, all rats were orally administered 1 mL of absolute ethanol. The animals were sacrificed by cervical dislocation 1 h following treatment, and their stomachs were once again removed, cut open along the greater curvature, and washed with saline solution. The ULI was determined as described above.

2.6. Protection from ulcerative lesions by prostaglandins

The participation of endogenous PG in the protection carried out by PepC or PepCF was ascertained via the following protocol: a 30 mg kg⁻¹ bw solution of indomethacin (I7378, from Sigma) was injected subcutaneously, as described by Szabo et al. (1981); 30 min afterwards, PepC, PepCF or 10 mL kg⁻¹ bw saline solution was administered. After another 30 min, the animals were given 1 mL of absolute ethanol orally, and sacrificed 1 h later. Their stomachs were removed and opened along the greater curvature, so as to once again calculate the ULI.

2.7. Protection from ulcerative lesions by nitric oxide

The role of NO on protection by PepC or PepCF was examined as follows: a 5 mg kg⁻¹ bw solution of N- ω -L-arginine methyl ester, L-NAME (N5751, Sigma) was administered through an

intraperitoneal route, as described by Konturek and Pawlik (1986). After 30 min, 10 mL kg⁻¹ bw PepC, PepCF or saline solution was administered orally; and after another 30 min, the rats received 1 mL of absolute ethanol orally. These animals were finally sacrificed 1 h later, and their stomachs removed and opened along the greater curvature, to permit assessment of lesions in terms of the ULI.

2.8. Statistical analyses

Analysis of variance (ANOVA) was applied to all experimental results, after preliminary confirmation of the homoscedasticity hypothesis. Differences among means were assessed by Duncan's test, at a significance level of 5%.

3. Results and discussion

3.1. Assessment of antiulcerogenic activity of whey protein hydrolysates

Administration of PepC in the range 30–350 mg kg⁻¹ bw according to the acute model, or of PepCF in the range 100–350 mg kg⁻¹ bw following the consecutive model, did not show any statistically significant dose/effect relationship in terms of ULI (Table 1), so DE₅₀ could not be calculated. A 10-fold increase of the extract concentration was not sufficient to significantly increase the active role of the whey peptides, or else the maximum activity had already been attained at 30 mg kg⁻¹ bw (i.e., the lowest concentration tested). This realization was not in agreement with the antiulcerative performance reported elsewhere for different doses of WPC and α -La, when ethanol (Matsumoto et al., 2001; Mezzaroba et al., 2006; Rosaneli et al., 2002) or indomethacin (Rosaneli et al., 2004) were employed. However, a similar absence of a dose/effect relationship was reported by Mezzaroba et al. (2006) in the case of α -La, using the indomethacin method. A likely explanation is that the bioactive peptides were made available in excess, so no limitation was apparent on the amount used (even at the lowest levels tested). However, some interference with the assay because of the lactose from whey may not be fully ruled out, since a complex mixture (rather than a pure compound) was used.

In view of the above results, an optimal dose of 100 mg kg⁻¹ bw was considered for the remaining tests that were aimed at ascertaining the cytoprotection mechanisms. In the acute model, the effectiveness of the aforementioned dose of PepCF and PepC, when compared with a single dose of 200 mg kg⁻¹ bw carbenoxolone, was measured as a decrease of ULI relative to its saline control. Carbenoxolone lowered ULI by 93.4%, whereas PepCF lowered it by only 68.5% and PepC by a mere 37.4%, so significant differences were observed ($p < 0.001$ for PepCF and carbenoxolone, and $p < 0.01$ for PepC). Therefore, PepCF was almost twice as effective as PepC, and approximately 66% as good as the reference drug.

Administration of three consecutive daily doses of 100 mg kg⁻¹ bw PepC led to results statistically similar to those of a single dose; however, PepCF was more efficient as a single dose, which led to approximately 70% inhibition, than as a triple dose which produced just approximately 30%; both of these were well below the inhibition caused by carbenoxolone, 93.4% (see Table 1). All treatments were significantly different ($p < 0.001$) from the negative control.

The peptides in PepC and PepCF were probably released by the two aspartic proteases in the flowers of *C. cardunculus* that have previously been described in terms of their significant and unusual proteolytic activity towards mainly α -La (Barros & Malcata, 2002, 2006; Lamas et al., 2000). However, this is the first time that an antiulcerogenic activity of the resulting whey peptides has been

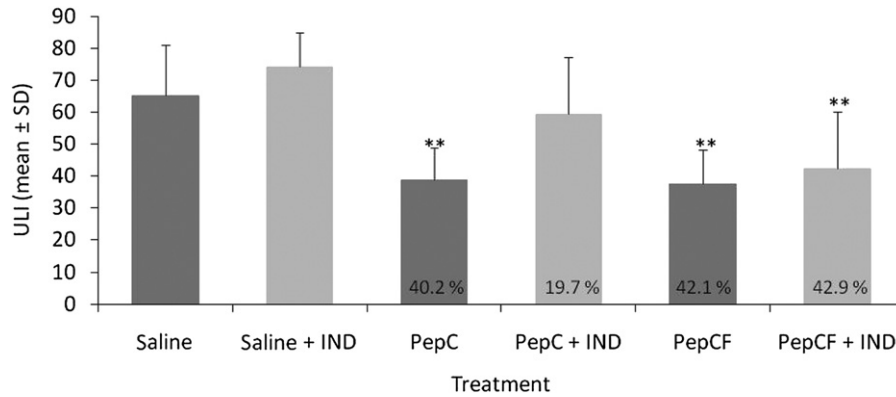


Fig. 1. Effects, in terms of the ulcerative lesions index, ULI (mean \pm standard deviation), of treatment with indomethacin (IND; an inhibitor of prostaglandin synthesis) administered by subcutaneous injection at $30 \text{ mg kg}^{-1} \text{ bw}$, regarding protection in rats against stomach ulcers induced by oral administration of ethanol by peptide concentrates PepC and PepCF, administered by intragastric intubation as a single dose of $100 \text{ mg kg}^{-1} \text{ bw}$ (with saline solution as negative control). The fractional decrease of ULI, based on saline or saline + IND, as appropriate, is also indicated.

observed. There is a distinct behaviour of cardosins upon the two dominant proteins in whey: α -La is highly susceptible to those enzymes, whereas β -lactoglobulin is not hydrolyzed to a significant extent (Barros & Malcata, 2006). This realization suggests that any peptides in WPC that may account for protection against gastric injury originated in α -La, which is in agreement with the claims by Matsumoto et al. (2001), Mezzaroba et al. (2006) and Ushida, Shimokawa, Toida, Matsui, and Takase (2007). On the other hand, previous studies encompassing α -La and its pancreatic hydrolyzate indicated a 32–50% decrease in ULI when two consecutive doses of $200 \text{ mg kg}^{-1} \text{ bw}$ were administered to rats (Matsumoto et al., 2001), whereas Mezzaroba et al. (2006) described an 82% reduction under similar conditions. If one considers α -La in WPC as the only precursor substrate able to protect against ulcerative lesions caused by ethanol, at an average concentration of 20% (w/w); and if this assumption is coupled with the protein concentration used in our study (37.4 and 68.5%, for PepC and PepCF, respectively), one finds that the reduction of ULI brought about by a $100 \text{ mg kg}^{-1} \text{ bw}$ dose of either PepC or PepCF is substantially higher than expected.

Inspection of the typical morphology of ethanol-induced gastric ulcers indicated that injury typically occurred on the mucosa in parallel to the long axis of the stomach, and mainly in the corpus compartments (with the antrum being less affected). The negative control underwent severe gastric injury, which was visible even from the outside of the stomach as thick red lines. Conversely, administration of PepC, PepCF or carbenoxolone (positive control)

reduced markedly the extent of injury. Therefore, our experimental results are particularly promising, as they suggest the existence of a protective effect conveyed by whey protein hydrolysates released by cardosins against ulcerative lesions caused by ethanol.

3.2. Postulation of cytoprotection mechanisms of whey protein hydrolysates

One underlying hypothesis for the protective effect of the whey protein hydrolysates against stomach ulceration is the content of sulphur-containing amino acids (or SH moieties). If this is large enough, it promotes biosynthesis of GSH, which in turn acts in conjunction with some PG as protective agent(s). However, such other amino acids as L-arginine could also be important – but via NO biosynthesis, since nitric oxide synthase, NOS, is known to also exhibit protective effects. Therefore, competitive absorption of low molecular weight, MW, peptides is favoured in the case of PepCF relative to PepC, so the biological activity of the former is expected to be higher.

To test whether the aforementioned mechanisms of protection of the gastric mucosa apply to our whey hydrolysates, the participation of SH moieties, PG and NO were specifically and separately addressed. The data in Figs. 1–3 accordingly represent the effects of blocking distinct metabolic routes, via specific agents, upon protection of the gastric mucosa by PepC and PepCF. Note that a saline solution and a specific blocking agent were always used as negative controls.

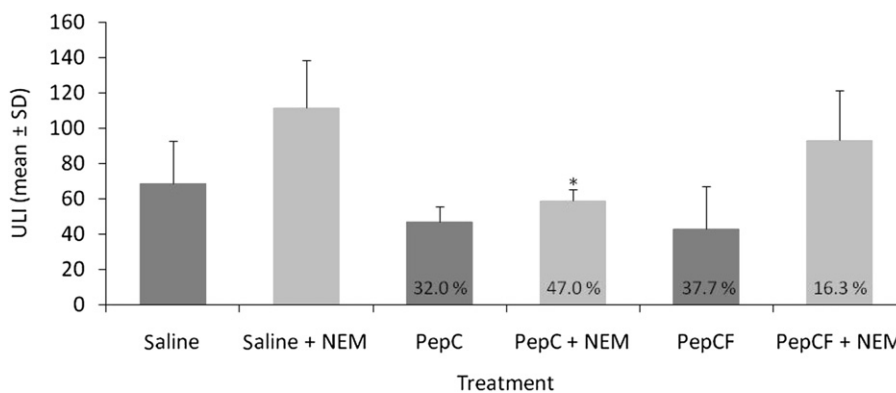


Fig. 2. Effects, in terms of the ulcerative lesions index, ULI (mean \pm standard deviation), of treatment with N-ethylmaleimide (NEM; an inhibitor of sulphhydryl compounds) administered by subcutaneous injection at $10 \text{ mg kg}^{-1} \text{ bw}$, regarding protection against stomach ulcers induced by oral administration of ethanol in rats by peptide concentrates PepC and PepCF, administered by intragastric intubation of a single dose of $100 \text{ mg kg}^{-1} \text{ bw}$ (with saline solution as negative control). The fractional decrease of ULI, based on saline or saline + NEM, as appropriate, is also indicated.

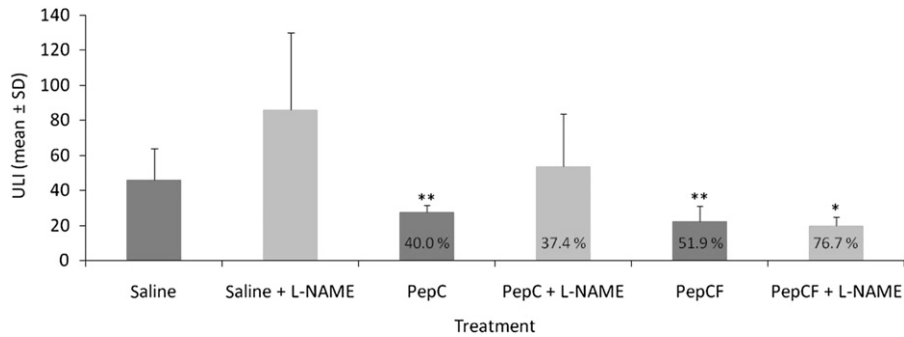


Fig. 3. Effects, in terms of the ulcerative lesions index, ULI (mean \pm standard deviation), of treatment with N- ω -L-arginine methyl ester (L-NAME; an inhibitor of NO synthesis) administered by peritoneal injection at 5 mg kg⁻¹ bw, regarding protection against stomach ulcers induced by oral administration of ethanol in rats by peptide concentrates PepC and PepCF, administered by intragastric intubation as a single dose of 100 mg kg⁻¹ bw. The fractional decrease of ULI, based on saline or saline + L-NAME, as appropriate, is also indicated.

Firstly, NEM was used to deactivate (via alkylation) all active SH groups in the rat's body; the treatment consisted in administration of 10 mg kg⁻¹ bw NEM via the intraperitoneal route, 30 min prior to the treatment with saline solution or whey protein hydrolysates. An increase in ULI was observed when the rats received NEM (Fig. 1), compared to those receiving only the saline solution. Alkylation of SH groups by NEM fully eliminated the protective effect brought about by PepCF; this is a strong clue for the high content of sulphur-containing amino acid residues of the whey protein hydrolysate being responsible for gastric protection. These amino acids can stimulate GSH synthesis (Sgarbieri, 1999), especially when they are part of small molecules as is the case of peptides, or even when they are free, as more likely happens with PepCF. The importance of SH compounds in protecting the stomach mucous membrane from the deleterious effects of ethanol has been emphasized elsewhere (Rosaneli et al., 2002, 2004; Szabo et al., 1981). However, a major portion (47%) of the antiulcerogenic activity of PepC remained (47%), even after quantitative blocking of -SH with NEM, suggesting that other mechanisms besides SH moieties were involved in the activity of this whey protein hydrolysate.

The gastric mucosa can be protected by PG through a variety of modes – viz. decrease of acid secretion, production of bicarbonate and mucus, and increase in blood flow (Miller, 1983; Terano, 1992; Wilson, 1991). Indomethacin was chosen to inhibit the cyclooxygenase involved in PG synthesis. The treatment consisted in administering 30 mg kg⁻¹ bw indomethacin via the intraperitoneal route, 30 min prior to the treatment with saline solution or whey protein hydrolysate. According to Fig. 1, an increase in ULI relative to the plain saline solution was observed in rats that had received indomethacin, so inhibition of PG hampered gastric protection. In the case of PepCF, treatment with indomethacin did not significantly affect its antiulcerogenic activity ($p < 0.05$) with regard to the negative control (43% protection). On the other hand, the protection provided by PepC was considerably dependent on PG; it dropped to just 19.7% upon administration of indomethacin. Therefore, the antiulcerogenic protective effect of PepC will mostly be accounted for by endogenous PG synthesis (Matsumoto et al., 2001).

Finally, endogenous NO enhances gastric cytoprotection by increasing mucosal blood flow, thus inhibiting release of oxygenated metabolites and proteases from leucocytes in the gastric microcirculation, besides modulating secretion of cellular growth factors, mainly epidermal growth factor (Elliot, Msknight, Cirino, & Wallace, 1995). L-NAME was thus used to inhibit NO synthetase, and thus disrupt the NO synthesis pathway. The treatment consisted in administering 5 mg kg⁻¹ bw L-NAME via the intraperitoneal route, 30 min prior to the treatment with saline solution or whey protein hydrolysates. Based on the results in Fig. 3, an increase in ULI was observed in rats after they had received L-NAME

relative to the plain saline solution; hence, inhibition of NOS hampered gastric protection. A similar result was observed when PepC was fed: the initially observed protective effect vanished in the presence of L-NAME, so the protective capacity by PepC may depend significantly on the NO level. In the case of PepCF treatment, L-NAME did not affect its antiulcerogenic activity to a significant degree, so NO does not appear to be actively involved. This extract did undergo an increase in its antiulcerogenic activity (approximately 78%, $p < 0.05$), so there is likely a large amount of L-arginine residues in PepCF. In any case, the dominant mechanism of action of PepCF appears to relate to SH groups.

4. Conclusions

The peptide concentrate (PepC), generated from whey by a set of less conventional plant proteases, and its fraction containing peptides with molecular mass below 3 kDa (PepCF), are both effective against ulcerative lesions of the gastric mucosa induced by oral administration of ethanol. The effectiveness of PepCF was almost 2-fold that of PepC: inhibition of ulcerative lesions ranged from 37.4% (PepC) to 68.5% (PepCF) in single-dose experiments of 100 mg kg⁻¹ bw – which compared with 93.4% by 200 mg kg⁻¹ bw carbenoxolone (taken as positive control). The most efficient protocol was an acute treatment; one single dose triggered a stronger effect than three consecutive daily doses.

In terms of metabolic pathways that might explain the observed protection of the stomach mucosa from ulcerative lesions, it appears that PepC stimulates PG and NO production, whereas PepCF depends on active SH compounds instead; however, these preliminary hypotheses need further validation. Clinical studies involving human volunteers are also required to confirm the promising results in this animal model system.

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