RESEARCH ARTICLE OPEN ACCESS

Effect of probiotic-fermented milk supplemented with carob pods on ethanol induced-ulcer in rats

Sana M'hir[1,2*], Kais Rtibi[3], Haifa habibi[3], Rym sassi[3], Lamia Ayed[1], Lamjed Marzouki[3], Hichem Sebai[3]

1 Laboratory of Ecology and Microbial Technology, National Institute of Applied Sciences and Technology (INSAT), Boulevard of the earth, BP 676 1080 Tunis, University of Carthage, Tunisia.
2 Department of Animal Biotechnology, Higher Institute of Biotechnology of Beja, University of Jendouba, 9000, BP: 382, Beja 9000, Tunisia
3 Laboratory of Functional Physiology and Valorization of Bio-resources, Higher Institute of Biotechnology of Béja, B.P. 382-9000, Béja, University of Jendouba, Tunisia.
*Corresponding author: Dr. Sana M'hir; E-mail : sana2617@yahoo.fr

Abstract:

The aim of this study was to test the effect of milks enriched with carob and fermented by two probiotic strains, separately, *Lactobacillus rhamnosus* LGG and *Lactobacillus casei* LCS, against ethanol-induced gastric ulcer in rats, as well as the associated disturbances. These beverages were orally administered to animals on a daily basis for one week. On day 7, two hours after the last treatment, the animals were intoxicated for one hour with a single dose of 80% ethanol. The famotidine was used as the positive control at a dose of 10 mg/kg and the untreated group received distilled water at 10 ml/kg. The animals were sacrificed and the stomachs were cut opened to observe and calculate the ulcer index. The ulcer index was determined and percentage of protection was calculated. The associated alterations in the gastric mucosa were analyzed by colorimetric methods. A single dose of ethanol (80%) caused major bleeding lesions and was accompanied by altered cellular mediators. However, the pretreatment of the animals with a mixture probiotic fermented milk and carob has led to a greater gastroprotective action than that observed after the individualized administration of these products. Mixture such carob and pobiotic can be suggested as functional food to protect against gastric ulcer.

Keywords — **Milk, carob, probiotic, functional fermented milk, gastric ulcer.**

I. INTRODUCTION

The ulcer is manifested by the loss of tissue substances from the skin or mucosal epithelial lining accompanied by damage to the underlying tissue planes. Common causes include the bacteria *Helicobacter pylori* and non-steroidal anti-inflammatory drugs (NSAIDs).

Other less common causes include tobacco smoking, stress due to serious illnesses, Behcet disease, Crohn disease and liver cirrhosis, among others [1, 2].

Carob is widespread in the Mediterranean basin [3,4]. According to the previous studies, carob flour had nutritional value and many researchers tried to

incorporate it as a functional food ingredient [5,6,7]. Studies have shown that carob reduce the risk of hypercholesterolemia [8]. Other studies have demonstrated its antioxidant properties *in vivo* and *in vitro* [9,10]. It also possesses an antidiarrheal and antidiabetic properties thanks to its richness in prebiotic potentialities [9]. Locust bean fibers also have an anticarcinogenic effect [11,12].

Probiotics are living microorganisms that, when administered in adequate amounts, are beneficial to the health of the host [13]. Prebiotics are non-digestible sugars and are part of dietary fiber. These dietary compounds, including galactooligosacharides (galactomanids in carob), stimulate the growth and / or activity of intestinal bacteria that have a positive impact on health. These functional probiotic foods have been the subject of scientific studies attempting to asseses their prospective potential [14].

The objective of this study is to evaluate the actions of probiotic-fermented milk supplemented with carob pods on ethanol induced-ulcer in rats.

II. MATERIEL AND METHODS

A. *Carob extract preparation*

The carob pods (collected from the Tabarka region, October 2020) were washed and dried (50°C for 72H) and then powdered in an electric blender (Moulinex Ovatio2, Fr). The carob extract was obtained by adding 1àg of powder in 100 mL of bidistilled water while stirring constanly (for 24h), then filtered to remove powdered precipitate and insoluble materials [15].

B. *Bacterial culture and fermented milk preparation*

Lactobacillus casei CS and *Lactobacillus rhamnosus* GG were grown separately in MRS broth at 37 °C. Bacteria were then harvested by centrifugation, washed twice with sterilized PBS (pH7.4) and resuspended in sterilized water. The resulting bacterial pellet is used to inoculate beverages. Viable bacteria numbers were determined by plate counts using MRS.

C. *Preparation of bacterial cultures enriched with carob*

FMCS-ca mixture: 10 g of milk powder (supplied by a dairy industry) were dissolved in 90 ml of distilled water. then, 10 ml of the supernatant obtained after the centrifugation of the aqueous extract of the carob are mixed with this dissolved milk. this mixture was pasteurized in a water bath at 95°c for 10 min. after cooling, the resulting drink is inoculated with *Lactobacillus casei*. Finally, the mixture was incubated at 37°c for 24 h.

FMGG-ca mixture: The same protocol described previously has been used. the resulting drink was inoculated with *Lactobacillus rhamnosus* and incubated at 37 ° C for 24 hours.

CS-ca mixture: 10 g of carob were hydrated with 100 ml of distilled water, pasteurized for 10 min at 95°C. After the cooling process, the obtained-solution was inoculated with *Lactobacillus casei* and then incubated at 37°C for 24 hours.

GG-ca mixture: The same protocol described previously has been used in this context and the resulting drink is inoculated with *Lactobacillus rhamnosus* and incubated at 37 °C for 24 hours.

FMGG: fermented milk with *Lactobacillus casei*

FMCS: fermented milk with *Lactobacillus rhamnosus*

D. *Animal treatment*

Wistar rats (weighting 200-220 g) were obtained from the Central Society of Pharmaceutical Industries of Tunisia (SIPHAT, Ben-Arours, Tunisia. The animals were divided into different separate groups and acclimatized for 15 days with a standard pellet diet (standard pellet diet Badr-Utique-TN) and water *ad libitum* (22 ± 2°C; 12 h dark/light cycle).

Sixty rats were separated into ten groups (n=6) and were treated orally as follows:

- Group 1 received orally 5 ml/kg of NaCl (0.9%) and served as a control group;
- Group 2 was intoxicated with ethanol (EtOH, 80%, 5 ml kg^{-1}, p.o.);
- Group 3 (FMCS-ca) was pre-treated with the mixture consisting of the fermented

mixture: milk, *Lactobacillus casei* (CS) and the aqueous extract of carob (10 ml/kg, p.o.);

- Group 4 (LGG-ca) of rats pretreated with the mixture consisting of the fermented mixture: milk, *Lactobacillus rhamnosus* (GG) and the aqueous extract of carob (10 ml/kg, p.o.);
- Group 5 (FMCS) of rats pretreated with milk fermented by *Lactobacillus casei* (CS) (10 ml/kg, p.o.);
- Group 6 (FMGG) of rats pretreated with milk fermented by *Lactobacillus rhamnosus* (GG) (10 ml/kg, p.o.);
- Group 7 (CS-ca) of rats pretreated with fermented carob and *Lactobacillus casei* (CS) (10 ml/kg, p.o.);
- Group 8 (GG-ca) of rats pretreated with fermented carob and *Lactobacillus rhamnosus* (10 ml/kg, p.o.);
- Group 9 (M) of rats pretreated only with milk (10 ml/kg, p.o.);
- Group 10 (ca) of rats pretreated with aqueous extract of carob at a dose of 10 ml/kg.

For one week, rats from the different groups received the different mixtures orally, while, the two other groups (C and EtOH) received physiological solutions. On day 7, two hours after the last treatment, the animals were intoxicated for one hour with a single dose of 80% ethanol (Table 2). The animals were subsequently sacrificed and the gastric mucosa was immediately removed and homogenized in a 50mM tris-NaCl buffer, pH 7.4. The supernatants thus obtained were used for biochemical assays.

E. *Gastric mucosal damage evaluation*

The stomach of each animal was removed and opened along its greater curvature. The issue was gently rinsed in NaCl 0.9%. the lesions in the gastric mucosa were macroscopically examined and the photographs of hemorrahagic erosions were acquired with a photometrics quantix digital camera. Ulcer indexes were determined as the sum of the lengths of the whole gastric lesions (in mm^2). Two independent, blinded observers performed the measurements of lesion lengths [16].

F. *Biochemical estimations*

The protein concentration was assessed according to the method reported by Hartree [17], which is a slight modification of the Lowry method. Serum albumin was used as the standard. The mucosa intestine H_2O_2 level was performed according to Dingeon *et al*., [18].

The mucosa intestine calcium level was performed using colorimetric method according to Stern and Lewis [19]. Briefly, at alkaline medium, calcium reacts with cresolphtalein leading to a colored complex measurable at 570nm.

The mucosa intestine non-haem iron was measured colorimetrically using ferrozine as described by Leardi *et al*., [20]. Concisely, the iron dissociated from transferrin-iron complex by a solution of guanidine acetate and reduced by ascorbic acid reacts with ferrozine to give a pink complex measured at 562nm.

G. *Stastistical test*

A one-way analysis of variance test was used to determine the significance between the different groups of all animals. Statistical analyses were calculated using StatView statistical software. The data are representative of six to eight distinct-observations. Differences were stated as mean ± SEM and designed significant when the values of *p* were inferior to 0.05.

III. RESULTS

A. *Microbiological enumeration*

Important growth of *L. casei* and *L. rhamnosus* are observed in the presence of milk (FMCS and FMGG, respectively). In the presence of carob extract only (CS-ca or GG-ca), this growth is about 7 $\log_{10}$ UFC/ml (Table 1).

No differences between *Lb casei* and *Lb rhamnosus* were observed for lactobacilli count in the mixture of fermented milk with carob extract (FMCS-ca or FMGG-ca). They reached a concentration of 7.94 ± 0.25 and 7.92 ± 0.25 log_{10} CFU/ml for *Lb casei* CS and *Lb rhamnosus* GG, respectively (Table 1).

TABLEAU I:

MICROBIAL ENUMERATION OF PROBIOTICS IN BEVERAGES AFTER FERMENTATION

Fermented beverages	Lactobacilli counts (log_{10} CFU/ml) t_{24h}
FMCS-ca	7.94^{c} ± 0.25
FMGG-ca	7.92^{c} ± 0.12
FMCS	7.9^{bc} ± 0.26
FMGG	7.23^{a} ± 0.19
CS-ca	7.41^{ab} ± 0.13
GG-ca	7^{a} ± 0.19

Means in a row followed by the same letter are not significantly different, p<0.05

B. *Effect of fermented beverages on ulcer development and mucus secretion*

The gavage of ethanol 80% in the rats caused a significant development of ulcer at the gastric level. In addition, the pretreatment of the animals with the carob enriched milk mixture and fermented by the probiotic bacteria LCS or LCG significantly decreased the extended surface area of the average ulcer (Table 2). Indeed, this area was considerably reduced compared to the positive group (Ethanol 80%). An ulcer index of 16.62 ± 1.22 mm^2 and 18.33 ± 1.67 mm^2 was observed for milks fermented by *Lactobacillus casei* and *Lactobacillus rhamnosus*, respectively. Gastroprotection is accompanied by a substantial increase in the secretion of gastric mucus. The volume of mucus is 3.6 ± 0.43 ml for *Lb rhamnosus* and 3.9±0.61 ml for *Lb casei.* This volume was 2.6 ± 0.33 mm^2 for the carob extract alone. It was only 2.1 ± 0.21 mm^2 for milk preparation. This gastroprotection is greater in the rats treated with the mixtures (LCS 74.92% and LCG 72.31%).

The macroscopic examination of gastric mucosa is shown in Fig. 1. As expected, EtOH administration exhibited injuries, including hemorrhage and hyperemia. In this respect, the results showed significant hemorrhagic lesions of a dark-red color in rats of the ethanol-group (Figure 1B) compared to the animals treated with physiological water or healthy stomach (Figure 1A). The stomachs (Figure 1I and 1J) belong to rats having received the fermented probiotic milks in association with the carob extract showed decreased lesions. The different administered drinks and famotidine treatment showed a dose-dependent decrease in all observed toxic signs compared with the EtOH treated group.

Moreover, quantitative analysis revealed that these beverages (FMCS-ca, FMGG-ca) or reference molecule pre-treatment significantly and dose-dependently decreased the ulcer index, increased the gastric volume juice, and improved the protection percentage of injury induced by EtOH administration .These improved results were much more noticeable following the administration of the mixtures.

C. *Effect of fermented beverages on biochemical parameters*

The treatment of the rats with ethanol (80%) caused a remarkable disruption of intracellular mediators such as hydrogen peroxide, free iron and calcium in the gastric mucosa compared to the control group (Table 3). These alterations were accompanied by an oxidation and a decrease in the level of gastric mucosal proteins in the group of intoxicated-rats with ethanol. In contrast, the pretreatment of these animals by carob, milk, probiotics (lactic acid bacteria) and mixtures induces a corrective action against all these changes. Indeed, this protection is more important in rats treated with probiotic fermented milks enriched with carob extract.

Tableau II:

EFFECT OF CAROB, MILK AND FERMENTED DRINKS ON THE DEVELOPMENT OF ULCER AND SECRETION OF MUCUS

Groups	Mucus Volume (ml)	Ulcer index (mm^2)	Percentage Protection (%)
A Control (10 ml/kg)	4.3 ± 0.70	-	-
B EtOH (5 ml/kg)	1.9 ± 0.20*	66.2 ± 2.6	-
C EtOH+ (M) (10 ml/kg)	2.1 ± 0.21*	63.2 ± 3.26	4.5
D EtOH+ Ca (10 ml/kg)	2.6 ± 0.33*#	55.2 ± 1.53#	16.6
E EtOH+ FMCS (10 ml/kg)	2.4 ± 0.04*	62.67 ± 2.66	5.33
F EtOH+ FMGG (10 ml/kg)	2.3 ± 0.12*	63.2 ± 2.81	4.5
G EtOH+ CS-ca(10 ml/kg)	2.9 ± 0.4*#	43.61 ± 4.21#	34.1
H EtOH+ GG-ca (10 ml/kg)	2.8 ± 0.31*#	46.2 ± 2.90#	30.13
I EtOH+ FMCS-ca (10 ml/kg)	3.9 ± 0.61#	16.62 ± 1.22#	74.92
J EtOH+ FMGG-ca (10 ml/kg)	3.6 ± 0.43#	18.33 ± 1.67#	72.31
Famotidine (10 ml/kg)	3.7 ± 0.45#	16.23 ± 3.12#	69.61

The results are expressed as mean ± SEM (n = 6). *: $p < 0.05$: the difference is significant compared to the control group. #: $p < 0.05$: the difference is significant compared to the ethanol group.

IV. Discussion

This study aims at evaluating the effect of the administration of aqueous carob extract, milk and fermented beverages by lactic acid bacteria on gastric ulcer and related disturbances. To the best of the authors' knowledge, the association of probiotic bacteria with carob fibers in a milk-based matrix has not been studied so far in the literature.

Anti-ulcer activity was tested on 80% ethanol-induced experimental ulcer in rats. The different protectors used in this manipulation were administered orally. The main cause of gastric ulcer is the destruction of the gastric mucosa. This destruction may be due either to an increase in gastric acid secretion or a decrease in mucus production and mucosal blood flow, leading to the erosion of the gastrointestinal wall [21]. Mucus can act therefore act as an antioxidant to reduce the damage caused by free radicals in the gastric mucosa [22].

In the present study, the administration of ethanol (80%) by gastric gavage in rats induced remarkable lesions of the gastric mucosa and a significant decrease in gastric mucus secretions. Contrarily, the pretreatment of animals with carob, milk, probiotics alone and fermented probiotic drinks induced significant protection of the gastric mucosa, especially following the administration of mixtures comparable to that of healthy controls. Indeed, the research of Lam *et al.*, [23] showed the role of

Lactobacillus rhanmnosus GG in restoring the ulcer for a dose of 10^9 CFU/day administered by gavage for 3 days. The area of the ulcer was decreased by 32%. In addition, this bacterium acts by stimulating angiogenesis through the production of metabolites and the deposition of peptidoglycan for the renewal

of damaged tissues [24]. This beneficial role has also been attributed to its probiotic power [25,26].

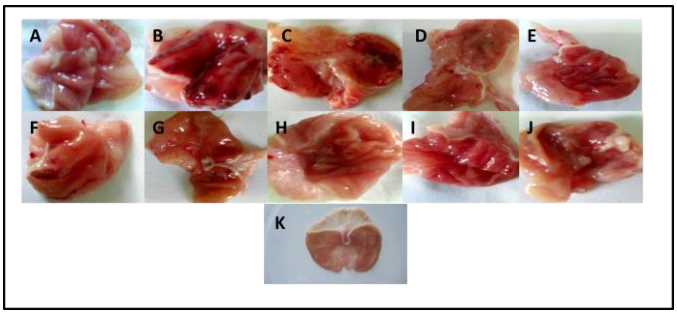

Fig. 1: Macroscopic analysis of ulcerated stomachs.
(**A**: control; **B**: EtOH; **C**: EtOH+ M; **D**: EtOH+ Ca; **E**: EtOH+ FMCS; **F**: EtOH+ FMGG;
G: EtOH+ CS-ca; **H**: EtOH+ GG-ca;
I: EtOH+ FMCS-ca; **J**: EtOH+ FMGG-ca;
K: Famotidine).

Added to that, Uchida and Kurakazu [27] evaluated the protective action of yoghurt containing *Lb gasseri* to restore HCl-induced ulcer. Culture supernatants of *Lb acidophilus* and *Bifidobacterium adolescentis* reduce ulcer formation [28]. This study has suggested the role of *Lactobacillus rhamnosus GG* as an alternative for the treatment of ulcers.
Clinical studies have reported the beneficial effect of probiotic bacteria [29]. Strains such as *Lb acidophilus* La-5 and *Bifidobacterium animalis subsp. lactis* BB-12 have been described in the treatment of ulcers [30]. A mixture of probiotic strains, with doses of 10^{11} and 10^{12} CFU/ml, made it possible to significantly reduce the ulcer [31].
Milks fermented with *Bifidobacterium* (1 x 10^{10} CFU/ml) were evaluated by [32]. Another aspect has been described by Rodrigues *et al*., [33] using *Lb casei* ATCC 393 encapsulated in microparticles of chitosan to inhibit *Hylocobacter pylori*.

The bacterium *Helicobacter pylori* was considered to be one of the most important development factors for gastric and duodenal ulcers [34].
The use of ethanol is a simple method for inducing experimental gastric ulcer in rats resulting from the alteration of the gastric mucosa. Ethanol thus shows its toxic effects through the direct generation of reactive metabolites, including free radicals and reactive oxygen species that react with most cell components causing the alteration of their structures and functions and therefore increased production of oxidative damage. Ethanol is metabolized in the body to release free radicals of superoxide and hydroperoxyl anions that are involved in the mechanism of acute and chronic ulceration of the gastric mucosa [35].

In the same context, our results showed that the treatment of rats with ethanol (80%) caused a significant increase in the production of H_2O_2 and a depletion of calcium and free iron levels. The pretreatment of the animals with the aqueous extract of carob, milk, probiotics and fermented beverages provided protection by the significant decrease in H_2O_2 associated with an increase in calcium and iron levels.
The various properties (antimicrobial, anti-inflammatory, etc.) of plant extracts are an asset for the use of these plants in the treatment of peptic ulcer. The potential of carob in the treatment of pathologies and in particular the ulcer is well mentioned by the research of Rtibi and collegues [36].

Tableau III:
EFFECT OF FERMENTED MILK, CAROB AND MIXTURE ON ETHANOL- INDUCED ULCER ASSOCIATED WITH BIOCHEMICAL PARAMETERS DISTURBANCES

Groups	Proteins (mg/mL)	H_2O_2 (mmol/mg proteins)	Calcium (µmol/mg proteins)	Iron (µmol/mg proteins)
Control (10 ml/kg)	24.65 ± 3.56	5.42 ± 0.35	18.00 ± 0.56	35.00 ± 4.27
EtOH (5ml/kg)	20.3 ± 2.45*	22.56 ± 3.61*	06.15 ± 0.26*	16.56 ± 2.45*
EtOH + M (10 ml/kg)	27.34 ± 3.12$^{*\#}$	20.45 ± 2.57*	20.38 ± 2.37$^{\#}$	24.56 ± 3.00$^{\#*}$
EtOH + Ca (10 ml/kg)	25.45 ± 4.83	17.45 ± 1.44*	17.56 ± 2.42$^{\#}$	16.53 ± 2.03*
EtOH + FMCS (10 ml/kg)	25.45 ± 2.37	18.56 ± 1.72*	13.47 ± 0.82$^{\#*}$	20.45± 2.74*
EtOH + FMGG (10 ml/kg)	24.34 ± 2.16	17.48 ± 0.71*	09.57 ± 0.28*	21.45 ± 0.35*
EtOH + CS-ca (10 ml/kg)	26.34 ± 3.55	15.33 ± 1.34*	14.46 ± 0.46$^{*\#}$	24.66 ± 3.46$^{\#*}$
EtOH + GG-ca (10 ml/kg)	25.45 ± 2.44	16.34 ± 0.90*	15.56 ± 0.46$^{\#}$	22.92 ± 2.40$^{\#*}$
EtOH + FMCS-ca (10 ml/ kg)	36.34 ± 5.74$^{*\#}$	12.35 ± 0.85$^{*\#}$	27.46 ± 3.56$^{*\#}$	40.26 ± 3.82$^{\#}$
EtOH + FMGG-ca (10 ml/ kg)	38.34 ± 3.85$^{*\#}$	16.34 ± 1.88*	20.46 ± 2.47$^{\#}$	36.36 ± 4.38$^{\#}$
Famotidine (10 ml :kg)	23.25 ± 1.23$^{\#}$	11.54 ± 1.33$^{*\#}$	16.49 ± 1.44$^{\#}$	32.90 ± 1.38$^{\#}$

The results are expressed as mean ± SEM (n = 6). *: $p < 0.05$: the difference is significant compared to the control group. $^{\#}$: $p < 0.05$: the difference is significant compared to the ethanol group.

This effect is most likely due to the phenolic compounds present in the pods of *Ceratonia siliqua*, which are known as antioxidant substances with the ability to trap radical species and reactive forms of oxygen. These compounds have antioxidant, chelation and anti-inflammatory activities. The pulp, known for its antioxidant activity [10] and can be used in the medicinal field, contains more pyrogallol and catechin than the seed. Catechin has antioxidant properties and may have a role in inhibiting the signaling pathway of cancer cells [37]. At this level, the cytoprotective role of antioxidants in the prevention and recovery of gastric lesions has been widely addressed in a number of studies [38].

Indeed, some ROS (reactive oxygen species) scavengers or inhibitors such as melatonin have protective effects against ethanol or indomethacin-induced gastric ulcers in rats [39].

Recently, Sistani *et al.* [40] reported that the induced ulcer inhibition in rats could be attributed to antioxidant properties and the ability to trap free radicals and reactive oxygen species. Thereby reducing the oxidative damage of the gastric mucosal membrane and epithelial cells.

Conversely, prebiotics stimulate the growth of probiotics. These ingested microorganisms in sufficient quantity, exert a beneficial effect on the health of the host. They contribute positively to the activity of the intestinal microflora and consequently to the health of the consumer [13]. The maintenance of this viability at the intestinal level uses substances of a polysaccharide nature approved under the name of prebiotics (inulin, fructo-oligosaccharides, galacto-oligosaccharides and lactulose).

Studies of prebiotics are less common than those of probiotics and their role in restoring ulcer lesions. Indeed, Furrie *et al.* [41] studied the combination of *Bifidobacterium longum* (2×10^{11} CFU/day) with inulin-oligofructose (6 g/day) for one month. Results are encouraging regarding the reduction of inflammation of the mucosa by cytokines.

V. Conclusion

Probiotics are very interesting when used in complements with an anti-ulcer treatment. Indeed, they improve its effectiveness and limit the digestive disorders that are most often associated. It appears that the probiotic fermented milk mixture and the aqueous extract of carob could be suggested for the development of nutraceutical foods. Our results open many perspectives about the use of probiotics associated with extracts of medicinal and aromatic plants as protective effect against induced ulcer.

ACKNOWLEDGEMENTS

Financial support of the Tunisian Ministry of Higher Education and Scientific Research (MHESR) is gratefully acknowledged.

REFERENCES

1. *K. Aziz,, D. Bonnet, and B. Foppa. "Hépato-gastro-entérologie. Paris : édition Masson ». 2éme édition. pp 322-323. 2012.*
2. *2. V.M. Cuevas, Y.R. Calzado, Y.P. Guerra, A.O. Year, S.J. Despaigne, R.M. Ferreiro, and D.C. Quintana. "Effects of grape seed extract, vitamin C, and vitamin e on ethanol- and aspirin-induced ulcers", Adv. Pharmacol. Sci. 740687. 2011*
3. *I. Battle, and J. Tous. "Carob Tree (Ceratonia Siliqua L.)", International Plant Genetic Resources Institute, Viadelle Sette Chiese, 142 00145, Rome, Italy. 1997.*
4. *S. Naghmouchi, M.L. Khouja, A. Romero, J. Tous and M. Boussaid. "Tunisian carob (Ceratonia siliqua L) populations: morphological variability of pods and kernel". Sci. Hortic., Vol. 121 (2), pp125–130. 2009*

.

5. *L. Sęczyk, M. Świeca, and U. Gawlik-Dziki, "Effect of carob (Ceratonia siliqua L.) flour on the antioxidant potential, nutritional quality and sensory characteristics of fortified durum wheat pasta", Food Chemistry, Vol. 194, pp 637–642. 2016.*
6. *S. Aydın, and Y. Özdemir. "Development and Characterization of Carob Flour Based Functional Spread for Increasing Use as Nutritious Snack for Children", Journal of Food Quality 5028150 , pp 1-7. 2017.*
7. *A. Radia, A. Boukhiar, K. Kechadi, and S. Benamara. "Preparation of a Natural Candy from Date (Phoenix dactylifera L.), Olive (Olea europaea L.), and Carob (Ceratonia siliqua L.) Fruits", Journal of Food Quality 9565931. 2018*
8. *H.J. Zunft, W. Luder, A. Harde, B. Haber, H.J. Graubaum, J. Gruenwald, "Carob pulp preparation for treatment of hypercholesterolemia", Advances in Therapy vol 18(5), pp 230–236. 2001.*
9. *B. Haber. "Carob fiber benefits and application". Cereal Foods World vol 47 (8), pp 365–369.202*
10. *H. Sebai, A. Souli, L. Chehimi , K. Rtibi , M. Amri, M. El-Benna , and M. Sakly, M.. "In vitro and in vivo antioxidant properties of Tunisian carob (Ceratonia siliqua L.)", Journal of Medicinal Plant Research vol 7(2), pp85–90. 2013.*
11. *. L. Corsi, R. Avallone, F. Cosenza , F. Farina, C. Baraldi, and M. Baraldi, M. "Antiproliferative effects of Ceratonia siliqua L. on mouse hepatocellular carcinoma cell line", Fitoter vol 73 (7-8), pp 674–684.2002.*
12. *S. Klenow, M. Glei, B. Haber, R. Owen, and B.L. Pool-Zobel. "Carob fibre compounds modulate parameters of cell growth differently in human ht29 colon adenocarcinoma cells than in LT97 colon adenoma cells", Food and Chemical Toxicology vol 46(4), 1389–1397. 2008.*
13. *Y.A. Ghouri, D.M. Richards, E.F. Rahimi, J.T. Krill, K.A. Jelinek, and A.W. DuPont. "Systematic review ofrandomized controlled trials of probiotics, prebiotics, and synbiotics in inflammatory bowel disease", Clinical and Experimental Gastroenterology vol 9(7), pp 473-87. 2014.*
14. *Derikx, L. A. A. P., Dieleman, L. A. and Hoentjen, F. 2016. "Probiotics and prebiotics in ulcerative colitis, Best Practice and Research", Clinical Gastroenterology vol. 30(1), pp 55-71.*
15. *K. Rtibi, M.A. Jabri, S. Selmi, A. Souli, H. Sebai, J. El-Benna, M. Amri, L. Marzouki, "Carob pods (Ceratonia siliqua L.) inhibit human neutrophils myeloperoxidase and*

in vitro ROS-scavenging activity", RSC Advances vol. 5(102), pp 84207-84215. 2015.

16. *D. Hollander, A. Taranawski, W.J. Krauseand and H. Gergely. "Protective effect of sucralfate against alcohol-induced gastric mucosal injury in the rat", Gastroenterology" 88(1 PT2), pp366–374. 1985.*

17. *E.F. Hartree. "Determination of protein: a modification of the Lowry method that gives a linear photometric response", Analytical Biochemistry vol 48(2), pp 422–7.1972.*

18. *B. Dingeon, J.P. Ferry, and A. Roullet. "Auto analysis of blood sugar by Trinder's method", Annales de Biologie Clinique vol 33(1), pp 3–13.1975.*

19. *J. Stern, and W.H. Lewis. "The colorimetric estimation of calcium in serum with ocresolphthalein complexone", Clinica Chimica Acta vol2, pp 576-80.1957.*

20. *A. Leardi, M. Caraglia, C. Selleri, S. Pepe, C. Pizzi, R. Notaro, A. Fabbrocini, S. De Lorenzo, M. Musicò, A. Abbruzzese, A.R. Bianco, and P. Tagliaferri. "Desferrioxamine increases iron depletion and apoptosis induced by ara-C of human myeloid leukaemic cells", British Journal of Haematology vol 102(3), pp 746–752.1998.*

21. *Z.A. T. Zakaria, Balan, A.K. Azemi, M.H. Omar, N. Mohtarrudin, Z. Ahmad, M.N. Abdullah, M.N. Desa, L.K. The, and M.Z. Salleh. "Mechanism(s) of action underlying the gastroprotective effect of ethyl acetate fraction obtained from the crude methanolic leaves extract of Muntingia calabura", BMC Complementary and Alternative Medicine vol16 pp 78. 2016.*

22. *N. Nazarbahjat, F.A. Kadir, A. Ariffin, M.A. Abdulla, Z. Abdullah, and W.A. Yehye. "Antioxidant Properties and Gastroprotective Effects of 2-(Ethylthio) Benzohydrazones on Ethanol-Induced Acute Gastric Mucosal Lesions in Rats", PLoS One. Vol 11(6), e0156022. 2016*

23. *E.K.Y. L.Yu. Lam, H.P.S. Wong, W.K.K. Wu, V.Y. Shin, E.K.K. Tai, W.H.L. So P.C.Y. Woo, and C.H. Cho. "Probiotic Lactobacillus rhamnosus GG enhances gastric ulcer healing in rats", European Journal of Pharmacology vol 565(1-3), pp 171–179. 2007.*

24. *D. Hirayama, T. Iida, and H. Nakase. " The Phagocytic Function of Macrophage-Enforcing Innate Immunity and Tissue Homeostasis", International Journal of Molecular Sciences vol 19(1) Pii:E92. 2017.*

25. *V.I. Nikitenko. "Infection prophylaxis of gunshot wounds using probiotics", Journal of Wound Care vol 13(9), pp363–366. 2004.*

26. *J.C. Valdez, M.C. Peral, M. Rachid, M. Santana, and G. Perdigon. "Interference of Lactobacillus plantarum with Pseudomonas aeruginosa in vitro and in infected burns: the potential use of probiotics in wound treatment", Clinical Microbiology and Infection Vol.11(6), pp 472–479. 2005*

27. *M. Uchida, and K. Kurakazu. "Yogurt containing Lactobacillus gasseri OLL2716 exerts gastroprotective action against acute gastric lesion and antral ulcer in rats". Journal of Pharmacological Sciences vol. 96(1), pp 84–90. 2004.2004.*

28. *G. Khoder, A.A. Al-Menhali, F. Al-Yassir, and S.M. Karam. "Potential role of probiotics in the management of gastric ulcer". Experimental and Therapeutic Medecine, vol 12(1), pp 3-17. 2016.*

29. *H.H. Cui, C.L. Chen, J.D. Wang, Y.J. Yang, Y. Cun, J.B.Wu , Y.H. Liu, H.L. Dan, Y.T. Jian, and X.Q. Chen. " Effects of probiotic on intestinal mucosa of patients with ulcerative colitis", World Journal of Gastroenterology vol 10(10), pp 1521-1525. 2004.*

30. *S. Wildt, I. Nordgaard, U. Hansen, E. Brockmann, and J.J. Rumessen. "A randomised double-blind placebo-controlled trial with Lactobacillus acidophilus La-5 and Bifidobacterium animalis subsp.lactis BB-12 for maintenance of remission in ulcerative colitis", Journal of Crohn's and Colitis vol 5(2), pp 115-21.2011.*

31. *R. D'Inca, M. Barollo, M. Scarpa, A.R. Grillo, P. Brun, M.G. Vettorato, I. Castaqliuolo, and G.C. Sturniolo. "Rectal administration of Lactobacillus casei DG modifies flora composition and Toll-like receptor expression in colonic mucosa of patients with mild ulcerative colitis". Digestive Diseases and Science, Vol. 56(4), pp1178-87. 2011.*

32. *K. Kato, S. Mizuno, Y. Umesak, Y. Ishii, M. Sugitani, A. Imaoka, M. Otsuka, O. Hasunuma, R. Kurihara, R. Iwasaki, and Y. Arakawa. 2004. "Randomized placebocontrolled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis", Alimentary Pharmacology and Therapeutics 20(10), pp: 1133-41. 2004.*

33. *F.J. Rodrigues, M.H. Omura, M.F. Cedran, F.H.F. Dekker, A.M. Barbosa-Dekker, and S. Garcia. "Effect of natural polymers on the survival of Lactobacillus casei encapsulated in alginate microspheres", Journal of Microencapsulation vol 34(5), pp: 431-439. 2017.*

34. *J.G. Kusters, A.H. Van Vliet and E.J. Kuipers. "Pathogenesis of Helicobacter pylori infection", Clinical Microbiology Reviews vol: 19(3), 449-90. 2006.*

35. *H. Hashim, F.F. Mughrabi, M. Ameen, H. Khaledi, and H.M. Ali. "Cytoprotective effect of benzyl N'-(5-chloro-indol-3-yl-methylidene)-hydrazinecarbodithioate against ethanol-induced gastric mucosal injury in rats", Molecules vol: 17(8), pp 9306-9320. 2012.*

36. *K. Rtibi, S. Selmi, M.A. Jabri, G. Mamadou, N. Limas-Nzouzi, H. Sebai, J. El-Benna, L. Marzouki,, B. Eto and Amri M. "Effects of aqueous extracts from Ceratonia siliqua L. pods on small intestinal motility in rats and jejunal permeability in mice", RSC Advances vol 6(50), pp 44345–44353. 2016.*

37. *C.S. Yang, H. Wang, J.X. Chen and J. Zhang. "Effects of Tea Catechins on Cancer Signaling Pathways", Enzymes vol 36 pp 195-221.2014.*

38. *M.H. Farzaei, M. Abdollahi, and R. Rahimi. "Role of dietary polyphenols in the management of peptic ulcer", World Journal of Gastroenterolology vol 21(21), pp 6499-517. 2015.*

39. *F.M. Birdane, M. Cemek, Y.O. Birdane, I. Gülçin, and M.E. Büyükokuroğlu.. "Beneficial effects of Foeniculum vulgare on ethanol-induced acute gastric mucosal injury in rats", World Journal of Gastroenterology Vol 13(4), pp: 607-611. 2007.*

40. *N. Sistani Karampour, A. Arzi, A. Rezaie, M. Pashmforoosh, and F. Kordi. "Gastroprotective Effect of Zingerone on Ethanol-Induced Gastric Ulcers in Rats". Medicina (Kaunas) vol 55(3): pii-E64. 2019.*

41. *E. Furrie, S. Macfarlane, A. Kennedy, J.H. Cummings, S.V. Walsh, D.A. O'Neil and G.T. Macfarlane. "Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial" Gut vol. 54(2), pp 242-249. 2015.*

Abbreviations

Ca: Carob

CS :*Lactobacillus casei*

CS-ca: *Lactobacillus casei*-carob

FMGG: Fermented Milk *Lactobacillus rhamnosus*

FMGG-ca: Fermented Milk *Lactobacillus casei*-carob

FMCS: Fermented Milk *Lactobacillus casei*

FMCS-ca: Fermented Milk *Lactobacillus casei*-carob

FM: Fermented milk

GG: *Lactobacillus rhamnosus*

GG-ca: *Lactobacillus rhamnosus*-carob

M: Milk

NSAIDs non-steroidal anti-inflammatory drugs

.