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Research Article

Benefits of MALLOLAX® on the Restoration of the Antioxidant Defenses in Intestinal Mucosal Barrier Depleted by Constipation

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Abstract

Background: Constipation is one of the most common functional gastrointestinal disorders in the general population, with a significant impact in the quality of life. It has been demonstrated that oxidative stress is involved in the etiopathogenesis of constipation. Furthermore, constipation can lead to oxidative injury, creating a 'vicious cycle'. A study on MALLOLAX®, a botanical extract derived from Malva sylvestris, with high concentrations on malvidin and malvidin-3-glucosides has been conducted in order to determine its antioxidant capacity and its potential on alleviating the constipation symptoms, and its positive influence on reverting the pathogenic mechanisms by protecting from oxidative stress.

Materials and methods: The antioxidant levels of MALLOLAX® were assessed at different concentrations using DPPH UV method. The antioxidant capacity was compared *versus* other botanical extracts commonly used in commercial supplements as senna and frangula extracts. Moreover, the interferences with loperamide the pharmaceutical constipation inductive substance were analyzed through both HPLC and UV method in order to assess the potential of MALLOLAX® in reverting the intestinal cells oxidation.

Results: MALLOLAX® showed higher antioxidant activity than other ingredients, being comparable to quercetin. Additionally, MALLOLAX® preserved the antioxidant capacity in presence of loperamide on a larger extent.

Conclusion: MALLOLAX® represents an excellent choice for alleviating constipation symptoms and has the potential to act through the underlying pathogenic mechanisms of the disorder, along with associated comorbidities, by protecting against the oxidative stress related to gastrointestinal disorders.

Keywords: Constipation; Oxidative stress; Malva sylvestris; Antioxidant; Loperamide

Introduction

Constipation is one of the most common functional Gastrointestinal (GI) disorders. Its etiology is multifactorial and diverse, leading to a heterogeneous range of symptoms that can vary among patients. It is characterized by persistently difficult and infrequent defecation, often accompanied by uncomfortable symptoms including dry stools, excessive straining, abdominal pain, incomplete evacuation and bloating [1,2]. Currently, the global prevalence of constipation, which significantly influences patients' quality of life and mental well-being, is estimated to be around 15% and increasing [2]. Constipation can be classified as either acute, typically lasting less than a week; or chronic [3]. The occurrence of chronic constipation is associated with various factors, which enable the classification of the disorder into primary and secondary causes [4].

Primary causes are associated with defects in the defecation process, the intestinal nervous system or the colonic function, or disruptions in bowel function resulting from lifestyle, eating patterns and dietary influence [3]. Secondary causes may include adverse drug reactions (e.g., opioid pain medication), systemic disorders (e.g., Parkinson's disease), and local colon pathologies (e.g., colorectal cancer) [3]. Constipation can lead to comorbidities, including hemorrhoids, anal fissures, rectal prolapse, stercoral ulcer… Moreover, several studies have suggested a possible link between constipation and colorectal cancer [5]. Two independent population-based studies have reported that chronic constipation is associated with increased mortality [6,7].

The role of oxidative stress in constipation, along with other GI diseases, has been investigated [8]. Prolonged constipation leads to oxidative injury and the potential destruction of free radicals. Inappropriate stimulation of the immune system repeatedly can result in an overproduction of free radicals and a reduction in the endogenous antioxidant system, creating an imbalance between the oxidant and antioxidant systems, thereby worsening oxidative stress [8].

Additionally, numerous studies have demonstrated that the imbalance between free radicals and antioxidants, as well as oxidative damage, plays a crucial role in the development of functional constipation. Moreover, oxidative stress leads to intestinal dysmotility,

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establishing a vicious cycle [9,10].

Jabri et al. [11] demonstrated in a recent study that oxidative stress is involved in the pathogenesis of constipation induced by loperamide. Inhibition of peristalsis was followed by oxidative damage, indicated by increased levels of Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), as well as by the reduction of sulfhydryl groups and glutathione. Furthermore, oxidative stress led to damage on the activity of colic antioxidant enzymes, including Catalase (CAT), Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD).

An association between chronic constipation and oxidative damage in children was showed in a clinical trial. Elevated levels of free radicals and Reactive Oxygen Species (ROS) can impact DNA directly, inhibiting or reducing DNA replication. These abnormally increased levels could also deactivate antioxidant molecules, such as vitamin C and E, SOD and CAT [12]. Moreover, prolonged constipation in children was found to lead to oxidative stress [13] and growth retardation [14], whereas effective management of constipation was observed to attenuate the growth retardation [14].

As it has been described, oxidative stress plays a role in the etiopathogenesis of several diseases associated with constipation [15]. The key question is whether relieving constipation can reduce oxidative stress and the risk of associated diseases, and there is evidence that suggests that this may indeed be the case [15]. Several mechanisms have been suggested above to elucidate how accelerating transit and preventing constipation can reduce oxidative stress.

Additionally, the intestinal microbiota may have the potential to significantly influence oxidative stress and development of constipation-associated diseases in the host. In an animal model, inducing constipation led to oxidative stress, while prebiotics reduced both constipation and oxidative stress [16].

MALLOLAX® is a natural extract obtained from Malva sylvestris harvested at Mediterranean soil. It contains more than 5% of polyphenols, including malvidin; as well as more than 30% of mucilages, which provide laxative properties. Malva sylvestris, commonly known as common mallow, has a long story of use in traditional medicine since 3000 BC [17]. The therapeutic effects are primarily associated with the leaves and flowers, because of larger concentrations of bioactive compounds, including flavonoids, mucilages, terpenoids, coumarins, sterols, tannins, alkaloids, saponins, and phenol derivatives [17-21]. Many pharmacological properties of Malva sylvestris are attributed to these compounds, such as antioxidant, anti-inflammatory, laxative, antibiotic, hepatoprotective and cytostatic properties [21].

Various studies revealed that the active ingredients of Malva sylvestris include mucilage, malvidin and its glucosides, malvin, tannins, antioxidants such as carotenoids or tocopherols, minerals and unsaturated fatty acids [20-22]. High molecular weight acidic polysaccharides located in the epidermal cells of the leaver were found to be of the rhamnogalacturonan type with glucose galactose and rhamnose as structural unit. The strong protective effect of Malva sylvestris extract on constipation has been studied extensively, with its effectiveness attributed not only to its antioxidant properties, but also to its ability to stimulate GI motility and intestinal secretion [11].

Considering the need for further research on the antioxidant capacity and laxative effect of Malva sylvestris extract, we conducted an investigation to assess the antioxidant properties of MALLOLAX® and its potential influence in constipation.

Materials and Methods

Chemicals and reactives

Chemicals and reactives used were: Metanol Panreac ACS grade, DPPH sigma Aldrich, Frangula Extract, Senna Extract, Quercetin, MALLOLAX®, UV-11, HPLC, loperamide.

Extract preparation

Aerial parts of Malva sylvestris were extracted using ethanol and water.

Analysis

The antioxidant properties of MALLOLAX® were compared to those of other botanical extracts known for their laxative effects: frangula and senna, as well as to antioxidant capacity of quercetin, used as a positive control. Furthermore, we examined the potential interaction between MALLOLAX® and the constipation-inducing agent loperamide.

Loperamide is an agonist of opioid receptors that inhibits gut peristalsis, decreases the propulsion of the intestinal contents, and increases the absorption of water and ions [23]. The main side effects of loperamide are bloating and constipation, and its cardiovascular toxicity has also been reported [24]. Loperamide has a direct influence in oxidation process and it is also used as the experimental model of constipation in rats.

Antioxidant properties were analyzed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method. Antioxidant levels of MALLOLAX®, MALLOLAX® at 37ºC and all the other botanicals were measured using the standard UV-Vis DPPH analysis [25]. MALLOLAX® levels were also measured using HPLC DPPH assay [26], in conjunction with loperamide for any possible interference.

UV-Vis DPPH analysis and quantification of MALLOLAX®, frangula, senna and quercetin: Samples were prepared at a concentration of 1000ppm, from which subsequent dilutions at 10, 50, 100, 250, 500 and 1000 ppm were performed. Sample dilutions were introduced in water at 37ºC for 4 hours. Posteriorly, DPPH was added to the solutions, allowed to react for 30 minutes. The outcomes were measured using UV at 517 nm.

UV-Vis DPPH analysis and quantification of MALLOLAX® and loperamide: MALLOLAX® samples were prepared at 1000 ppm, from which dilutions were made at 100 and 250 ppm. Loperamide samples were prepared at 500 ppm, from which subsequent dilutions at 100 and 250 ppm were prepared. A DPPH solution was also prepared. Absorbance was measured at 517 nm, and a spectrum scan was conducted in the range of 200 nm-600 nm.

HPLC DPPH analysis and quantification of MALLOLAX® and loperamide: MALLOLAX® samples were prepared at 1000 ppm, from which dilutions were made at 100 ppm and 250 ppm. Loperamide samples were prepared at 500 ppm, with subsequent dilutions at 100 ppm and 250 ppm. A DPPH solution was also prepared. The reaction was carried out in amber HPLC vials, incubated for 20 minutes. The chromatographic analysis was performed at 517 nm, and a spectrum scan was conducted in the range of 200 nm-600 nm.

Results

UV-Vis DPPH analysis and quantification of MALLOLAX®, frangula, senna and quercetin

Table 1 and Figure 1

Table 1: DPPH radical percentages of each botanical determined by UV-vis spectroscopy.

% of Inhibition by UV-Vis analysis					
Sample	**MALLOLAX®**	**MALLOLAX® 37°C**	**QUERCETIN**	**FRANGULA**	**SENNA**
10 PPM	43	44	92	36	34
50 PPM	71	72	92	46	44
100 PPM	90	91	92	54	55
250 PPM	91	91	92	69	74
500 PPM	90	90	92	79	72
1000 PPM	90	88	92	73	52

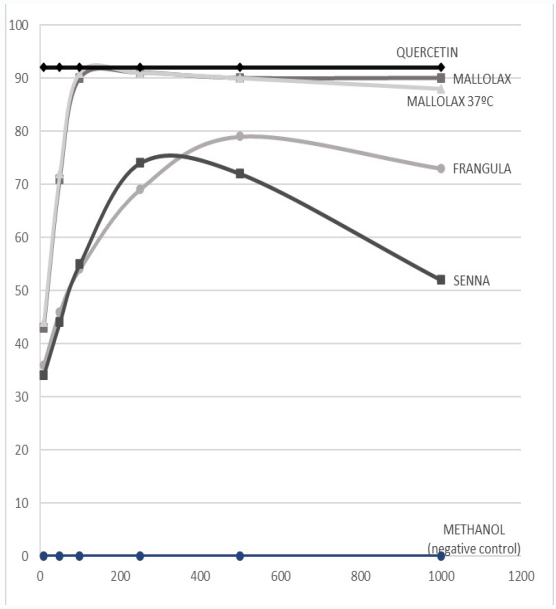

Figure 1: Comparation of the radical DPPH inhibition percentages of each MALLOLAX®, senna and frangula determined by UV-Vis at different range of concentrations (50-1000 ppm).

UV-Vis DPPH analysis and quantification of MALLOLAX® and loperamide

Table 2

HPLC DPPH analysis and quantification of MALLOLAX® and loperamide

Table 3 and Figure 2

Results obtained from UV and HPLC were comparable.

Discussion

Constipation is one of the most common GI disorders, characterized by difficult and infrequent defecation, often accompanied by uncomfortable symptoms, such as dry stools, excessive straining, abdominal pain, incomplete evacuation and bloating [1,2].

Persistent constipation can lead to oxidative damage and potential destruction due to free radicals, creating an imbalance between the oxidant and antioxidant systems, thus worsening oxidative stress [8]. Inhibition of peristalsis was followed by oxidative damage, resulting in increased levels of MDA and H_2O_2 and the reduction of sulfhydryl groups and glutathione. Oxidative stress also caused damage on the activity of colonic antioxidant enzymes, including CAT, GPx and SOD [11].

MALLOLAX® is a natural extract obtained from Malva sylvestris, known for its potential medical applications in constipation, particularly due to its mucilage content. It also contains various biologically active compounds, including flavonoids, tannins and anthocyanins. Malva sylvestris, among other therapeutic uses and properties, exhibits a laxative effect by the stimulation of GI motility and intestinal secretion [11]. In this study, we tried to demonstrate its antioxidant properties, which could provide an additional advantage in constipation treatment.

Several clinical studies have investigated the effectiveness of Malva sylvestris in the treatment of constipation. In a placebo-controlled trial, researchers evaluated the laxative effect of Malva sylvestris in patients with constipation. The study involved 110 participants, who were randomly assigned to receive either Malva sylvestris or a placebo. The results showed that the group that received Malva sylvestris experienced a significant improvement in their bowel movements and in symptomatology of constipation, with an increase in stool frequency and a decrease in stool hardness compared to the placebo group [27].

The laxative effect of Malva sylvestris is attributed to its high mucilage content, which acts as a bulk-forming agent that increases the water content of the stool, softening it and making it easier to pass. Additionally, Malva sylvestris has been shown to have a prokinetic effect on the GI tract, stimulating the movement of food and waste through the digestive system [11].

Malvidin, one of the components of Malva sylvestris extract, appears to play a key role in the antioxidant capacity of MALLOLAX®. Malvidin is a food anthocyanin that possesses brilliant antioxidant properties in vitro. In nature, malvidin is typically found in two different glycosylated forms at the C-3 position: malvidin-3-glucoside and malvidin-3-galactoside. These glycosides present a significantly enhanced antioxidant effect compared to malvidin itself [27]. This molecule is a strong antioxidant due to its multiple hydrogen-bond donors, which enable malvidin to scavenge ROS effectively [28,29]. Malvidin presents a purple tonality and it can be found in various fruits, including a wide variety of grapes [30], blueberries [28], and blue-colored flowers [31].

The stable free radical DPPH was used to determine the radical

Table 2: DPPH radical percentages of MALLOLAX®, loperamide and MALLOLAX® + loperamide determined by UV-vis spectroscopy.

% of Inhibition UV-Vis analysis			
Sample	**MALLOLAX®**	**LOPERAMIDE**	**MALLOLAX® + LOPERAMIDE**
100 ppm	91	34	79
250 ppm	92	33	91

Table 3: DPPH radical percentages of MALLOLAX®, loperamide and MALLOLAX® + loperamide determined by HPLC analysis.

% of Inhibition HPLC analysis			
Sample	**MALLOLAX®**	**LOPERAMIDE**	**MALLOLAX® + LOPERAMIDE**
100 ppm	75	2	50
250 ppm	100	0	97

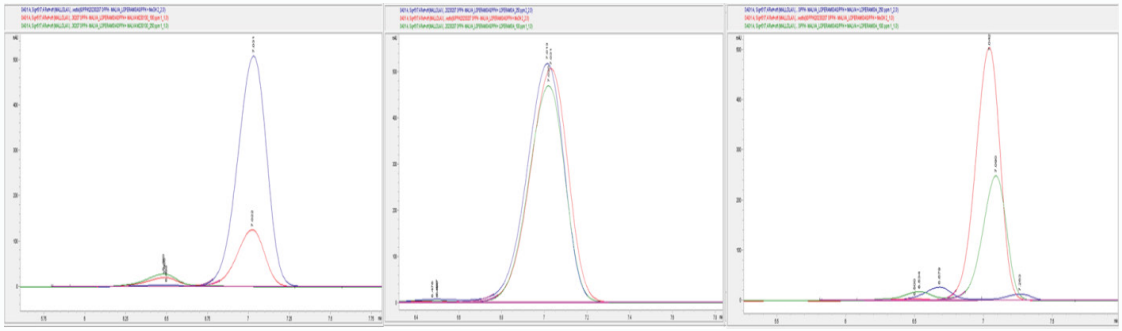

Figure 2: HPLC results of MALLOLAX®, loperamide and MALLOLAX® + loperamide, respectively.

scavenging properties of botanicals confirming the potentiality of the bioactive components from MALLOLAX® to act as donors of hydrogen atoms. The results obtained showed that MALLOLAX® possess an elevated antioxidant activity with IC50=.35 ± .01 mg/mL compared to different botanical extracts. The protective effect of MALLOLAX® against free radicals might be caused mostly by the activity of malvidin and malvidin-3-glucoside molecules.

Test results of DPPH radical scavenging activity measured by UV-Vis spectroscopy method on frangula, senna and MALLOLAX® are summarized in Figure 1. The data revealed that MALLOLAX® was the most efficient substance in protecting against free radicals, which are considered potential inducers of constipation, and, at the same time, a negative consequence of the disorder.

Furthermore, results obtained by UV-Vis and HPLC, summarized in Tables 2 and 3 respectively, indicated that loperamide, used as an antidiarrheic and as the experimental model of constipation in rats, just partially inhibits the antioxidant activity of MALLOLAX®. Through this study we demonstrated that loperamide is also prooxidative stress mediated by constipation-inducer at 100 ppm. However, MALLOLAX® at 250 ppm fully reverts the intestinal mucosal barrier depleted by constipation.

Therefore, MALLOLAX® constitutes a promising option as an antioxidant, as its capacity is higher than other commonly used laxative botanicals. The supplementation of MALLOLAX® could represent an improvement in addressing oxidative stress generated by constipation and in quality of life.

Conclusion

These results demonstrate that MALLOLAX®, a Malva sylvestris natural extract obtained from flowers and leaves, constitutes an excellent option in the constipation treatment, due to the osmotic activity from mucilages and also to the antioxidant properties of the anthocyanidins malvidin and malvidin-3-glucoside, which hold the potential to contribute positively to managing the underlying pathogenic mechanism of constipation by protecting against oxidative stress. Nevertheless, further investigation is necessary to completely understand the antioxidant capacity of Malva sylvestris extract and its relation with constipation.

References

1. Rao SSC, Rattanakovit K, Patcharatrakul T. Diagnosis and management of chronic constipation in adults. Nat Rev Gastroenterol Hepatol. 2016;13(5):295-305.
2. Bharucha AE, Lacy BE. Mechanisms, Evaluation, and Management of Chronic Constipation. Gastroenterology. 2020;158(5):1232-49.e3.
3. Camilleri M, Ford AC, Mawe GM, Dinning PG, Rao SS, Chey WD, et al. Chronic constipation. Nat Rev Dis Primer. 2017;3(1):1-19.
4. Andrews CN, Storr M. The pathophysiology of chronic constipation. Can J Gastroenterol. 2011;25(Suppl B):16B-21B.
5. Talley NJ, Lasch KL, Baum CL. A Gap in Our Understanding: Chronic Constipation and Its Comorbid Conditions. Clin Gastroenterol Hepatol. 2009;7(1):9-19.
6. Koloski NA, Jones M, Wai R, Gill RS, Byles J, Talley NJ. Impact of persistent constipation on health-related quality of life and mortality in older community-dwelling women. Am J Gastroenterol. 2013;108(7):1152-8.
7. Chang JY, Locke GR, McNally MA, Halder SL, Schleck CD, Zinsmeister AR, et al. Impact of functional gastrointestinal disorders on survival in the community. Am J Gastroenterol. 2010;105(4):822-32.
8. Mousavi T, Hadizadeh N, Nikfar S, Abdollahi M. Drug discovery strategies for modulating oxidative stress in gastrointestinal disorders. Expert Opin Drug Discov. 2020;15(11):1309-41.
9. Kashyap P, Farrugia G. Oxidative Stress: Key player in Gastrointestinal Complications of Diabetes. Neurogastroenterol Motil. 2011;23(2):111-4.
10. Talley NJ. Letter: chronic constipation - a warning sign for oxidative stress? Author's reply. Aliment Pharmacol Ther. 2015;42(3):386.
11. Jabri MA, Wannes D, Hajji N, Sakly M, Marzouki L, Sebai H. Role of laxative and antioxidant properties of Malva sylvestris leaves in constipation treatment. Biomed Pharmacother. 2017;89:29-35.
12. Wang JY, Wang YL, Zhou SL, Zhou JF. May chronic childhood constipation cause oxidative stress and potential free radical damage to children? Biomed Environ Sci. 2004;17(3):266-72.
13. Zhou JF, Lou JG, Zhou SL, Wang JY. Potential oxidative stress in children with chronic constipation. World J Gastroenterol. 2005;11(3):368-71.
14. Chao HC, Chen SY, Chen CC, Chang KW, Kong MS, Lai MW, et al. The Impact of Constipation on Growth in Children. Pediatr Res. 2008;64(3):308-11.
15. Vermorken A, Andrès E, Cui Y. Letter: chronic constipation - a warning sign for oxidative stress? Aliment Pharmacol Ther. 2015;42(3):385-6.
16. Li Y, Zong Y, Qi J, Liu K. Prebiotics and Oxidative Stress in Constipated Rats. J Pediatr Gastroenterol Nutr. 2011;53(4):447-52.
17. Gasparetto JC, Martins CAF, Hayashi SS, Otuky MF, Pontarolo R. Ethnobotanical and scientific aspects of Malva sylvestris L.: a millennial herbal medicine. J Pharm Pharmacol. 2012;64(2):172-89.
18. Classen B, Blaschek W. An Arabinogalactan-Protein from Cell Culture of Malva sylvestris. Planta Med. 2002;68(3):232-6.
19. Billeter M, Meier B, Sticher O. 8-hydroxyflavonoid glucuronides from Malva sylvestris. Phytochemistry. 1991;30(3):987-90.
20. Barros L, Carvalho AM, Ferreira ICFR. Leaves, flowers, immature fruits and leafy flowered stems of Malva sylvestris: A comparative study of the nutraceutical potential and composition. Food Chem Toxicol. 2010;48(6):1466-72.
21. Batiha GES, Tene ST, Teibo JO, Shaheen HM, Oluwatoba OS, Teibo TKA, et al. The

phytochemical profiling, pharmacological activities, and safety of malva sylvestris: a review. Naunyn Schmiedebergs Arch Pharmacol. 2023;396(3):421-40.

22. Tomoda M, Gonda R, Shimizu N, Yamada H. Plant mucilages. XLII. An anti-complementary mucilage from the leaves of Malva sylvestris var. mauritiana. Chem Pharm Bull (Tokyo). 1989;37(11):3029-32.

23. Basilisco G, Bozzani A, Camboni G, Recchia M, Quatrini M, Conte D, et al. Effect of loperamide and naloxone on mouth-to-caecum transit time evaluated by lactulose hydrogen breath test. Gut. 1985;26(7):700-3.

24. Eggleston W, Palmer R, Dubé PA, Thornton S, Stolbach A, Calello DP, et al. Loperamide toxicity: recommendations for patient monitoring and management. Clin Toxicol (Phila). 2020;58(5):355-9.

25. Gulcin İ, Alwasel SH. DPPH Radical Scavenging Assay. Processes. 2023;11(8):2248.

26. Marañon JA, de los Santos L, Lozano C, Martinez-Campesino L. Solgen 40, the high Genistin/ Genistein soy concentrate prevents bone mineral loss during menopause in a dose concentrate manner preventing the oxidized bone microenvironment. 2015.

27. Elsagh M, Fartookzadeh MR, Kamalinejad M, Anushiravani M, Feizi A, Behbahani FA, et al. Efficacy of the Malva sylvestris L. flowers aqueous extract for functional constipation: A placebo-controlled trial. Complement Ther Clin Pract. 2015;21(2):105-11.

28. Huang W, Zhu Y, Li C, Sui Z, Min W. Effect of Blueberry Anthocyanins Malvidin and Glycosides on the Antioxidant Properties in Endothelial Cells. Oxid Med Cell Longev. 2016;2016:e1591803.

29. Chorfa N, Savard S, Belkacemi K. An efficient method for high-purity anthocyanin isomers isolation from wild blueberries and their radical scavenging activity. Food Chem. 2016;197 Pt B:1226-34.

30. Mazza G, Francis FJ. Anthocyanins in grapes and grape products. Crit Rev Food Sci Nutr. 1995;35(4):341-71.

31. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res. 2017;61(1):1361779.