Efficacy of oral intake of a compound medical device in the treatment of laryngopharyngeal reflux disease: a clinical investigation and nasal cytological correlations

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To the Editor,

Laryngopharyngeal reflux (LPR) is a condition characterized by gastroduodenal contents rising up the oesophagus, thus coming into contact with the epithelium lining the larynx and hypopharynx (1, 2). Typical manifestations of LPR include raclage, asthma, chronic cough, dysphonia, hypopharyngeal globus sensation and laryngitis (2). In some cases, gastric contents may reach the nasal cavities, inducing or exacerbating rhinitis and sinusitis (2-4). Clinical studies have shown that LPR has a negative effect on nasal resistance and nasal congestion and that treatment of LPR can lead to subjective and objective improvement in nasal complaints (5). The chronic inflammatory process that characterizes LPR leads to an increase in the presence of inflammatory cells, and a progressive remodelling of the nasal mucosa, until the manifestation of goblet cell metaplasia. This situation leads to a progressive impairment of mucociliary clearance, which, in turn, leads to bacterial colonization and the onset of infections, which further support the inflammatory state; this gives rise to a vicious circle with mutual reinforcement of inflammation and metaplasia. Nasal cytology represents a useful, inexpensive and easily applicable diagnostic method that allows, through the quantification of cell populations in the nasal mucosa, to detail the phenotypic characteristics of LPR to better discriminate pathological conditions and assess pathological conditions the effect of the applied therapeutic strategy. A recent study confirmed the relationship between nasal mucosal cytology, chronic rhinosinusitis and gastroesophageal reflux disease by comparing the results of rhinocytogram and pH-impedance testing (4). Suppression of gastric acid production by proton pump inhibitors (PPIs) remains the mainstay of LPR treatment, although the efficacy of PPIs in the treatment of LPR is controversial (6). The objectives of this study are to assess the efficacy of the compound medical device in reducing the signs and symptoms of LPR and in protecting the mucosa of the upper respiratory tract from the potential damage of gastric reflux, also by performing a comparison between the state of the nasal mucosa of healthy subjects and the state of the nasal mucosa of patients suffering from LPR.

MATERIALS AND METHODS

Medical device tested

Leniref® (Lmd; Pharma Line S.r.l. Milan) is a compound medical device (EC certificate no. 471-00-00 DM, obtained on 20 April 2020) based on magnesium alginate, calcium carbonate, potassium bicarbonate, extract of *Opuntia ficus-indica* L. and extract of *Olea europaea* L. The product is indicated for the treatment of gastroesophageal reflux and

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associated symptoms. It is also indicated for the treatment of LPR and its associated symptoms. The product was provided free of charge by Pharma Line (Milan).

Subjects assessed

Sixty-eight subjects were assessed, including 41 patients (23 men, 18 women) with LPR and treated with Lmd, and 27 healthy subjects (12 men, 15 women). The demographic and medical history data of the patients enrolled in the study are shown in Table I.

Adult male and female patients with LPR were included in the study. To be included, patients had to be older than 18 years, having symptoms of LPR for at least 3 months and at least 3 times a week, score on the Reflux Symptom Index (RSI) greater than 13, and at video-laryngoscopy have morphological lesions of the larynx attributable to LPR, as evidenced by a Reflux Finding Score (RFS) greater than 7. Patients who had not undertaken continuous treatment yet for LPR, or those who had already had treatment for LPR, were enrolled. In the latter case, T0 was 15 days after discontinuation of any therapy based on antacids, alginates and related products. Patients already treated chronically with PPIs or H2-receptor antagonists maintained a constant drug dosage throughout the study period.

Subjects with known sensitivity to one or more components of Lmd, malignant or inflammatory diseases of the upper respiratory tract and upper gastrointestinal tract, inhalant or food allergies and pregnant and breastfeeding women were excluded from the study.

Twenty-seven healthy subjects were also enrolled for comparison purposes. These subjects were required to have an RSI score of less than 13 and no morphological lesions of the larynx attributable to LPR at video-laryngoscopy, which was confirmed by an RFS score of less than 7.

Subjects were enlisted with their identification data and signed regular informed consent to both the proposed therapy and the processing of personal data. At the time of enrolment, a medical history form was completed for each patient with the data collected, and a second form was provided to be returned on their next check-up.

Study design

This prospective, multicentre, observational study compares LPR patients treated with the medical device with healthy subjects. The period of patient enrolment and treatment was extended from February to August 2021.

Patients in the Lmd group were screened on three occasions: at the start of Lmd intake (T0), after 30 days of Lmd intake (T1) and after 30 days of stopping Lmd intake (T2). From the time of initiation (T0) and for the following 30 days (T0 to T1), patients took one sachet of Lmd after the two main meals and before going to bed. In addition, healthy subjects were tested on one occasion (T0).

Assessment

At the time of enrolment, subjects underwent an accurate

Table I. *The demographic and medical history data of the patients enrolled in the study and healthy subjects compared. Data are expressed as mean ± SD, unless otherwise stated.*

Variable	Patients treated (n=41)	Healthy subjects (n=27)
Age in years	52.4 ± 13.1	31.6 ± 10.0
Gender, % F (n)	43.9 (18)	56.6 (15)
Height (cm)	169.7 ± 7.6	169.2 ± 7.2
Weight (kg)	68.2 ± 13.2	64.5 ± 10.7
BMI	23.6 ± 3.4	22.4 ± 2.5
Smoking, % yes (n)	31.7 (13)	29.6 (8)
Presence of symptoms since (months)	28.9 ± 46.6	
Days with symptoms per week	5.6 ± 1.8	
PPI intake, % yes (n)	34.1 (14)	

medical history with an assessment of symptoms, allergies, smoking, occupation, familiarity with allergic and non-allergic nasal diseases, operations undertaken and ongoing therapies. Objective examination and rhinocytogram were also conducted. For the latter, all LPR patients were sampled using the scraping technique at T0, T1 and T2, whilst healthy subjects were sampled once (T0). The sample was spread on a slide and stained using the May Grunwald - Giemsa method. The prepared slides were observed at 100, 400, 1000 magnifications, counting the inflammatory cellular elements (neutrophils and lymphocytes), goblet cells and any bacteria present. The outcome of the nasal cytological examination was assessed using the classification shown in Table II (7, 8).

Patients with LPR at T0, T1 and T2 and healthy subjects at T0 only answered the questions on the RSI questionnaire and underwent video-laryngoscopy, whereby the investigating physician completed the RFS index.

Patients were also asked to rate the effectiveness of their treatment using a numerical rating scale (NRS) numbered from 0 (no effectiveness) to 10 (maximum possible effectiveness).

A sample of 23 patients treated with Lmd and 15 healthy subjects was evaluated by using, as a method of symptom detection, the Total Nasal Symptom Score (TNSS), which assigns a 4-point rating (from 0 to 3), depending on the presence and intensity of the symptoms the patient is experiencing, among the following 4 nasal symptoms: nasal obstruction, rhinorrhoea, nasal itching and sneezing. Lastly, reports of any adverse effects attributable to taking Lmd were collected.

Statistical analysis

Descriptive statistics were used to summarise the characteristics of the cohorts in terms of median, mean and standard deviation (SD) or frequencies when appropriate.

The treatment effect was estimated in terms of change in outcome in treated patients between the T1 and T2 visit and the T0 visit and in terms of change in outcome between treated patients at the three visits and healthy subjects. The significance of the differences was determined by applying the non-parametric Mann-Whitney test for paired data of treated patients in the case of the comparison between T2, T1 and T0 and for unpaired data in the case of the comparison of changes between treated patients and healthy subjects. In all the analyses carried out, the results

Table II. *Quantitative classification of nasal cytology results (*average of cells per 10 fields at high magnification - 1000x; from [(8), modified].*

Cellular element	Description	Quantity	Classification
Neutrophils, eosinophils and lymphocytes	None	0*	0
	Sporadic	0.1 – 1.0*	½+
	A few scattered cells, small groups	1.1 – 5.0*	1+
	In discreet numbers, large groups	5.1 – 15*	2+
	Large cellular clusters that do not occupy the entire field	15.1 – 20*	3+
	Large cellular clusters occupying the entire field	> 20*	4+
Goblet cells	None	0	0
	From rare to few cells	1 -24%	1+
	In a significant number	25 - 49%	2+
	In large numbers	50 - 74%	3+
	Lots of cells scattered all over the field	75 – 100%	4+
Bacteria	None	Normal / pathological	0
	Sporadic cell cluster		1+
	In a significant number		2+
	Many easily visible cells		3+
	Bacteria in the entire field		4+

are considered statistically significant for $p < 0.05$.

Statistical analysis was performed using the R software version 3.6.1 for Windows (R Core Team; 2013. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

On average, patients were evaluated 29.5 ± 2.0 days (T1) after T0 and 28.5 ± 3.8 days (T2) after T1. However, one patient did not complete the study intake cycle and was excluded from the results. Analyses were then conducted using data from the remaining 40 patients.

In patients with LPR, several signs and symptoms can affect the mucous membranes of the upper respiratory tract. In our study, swelling and congestion of the posterior laryngeal mucosa, especially of the posterior thirds of the vocal cords and of the arytenoids, hypertrophic lesions of the posterior laryngeal commissure and a discrete presence of mucus presented most commonly in the enrolled LPR patients. Cytologically, in the nasal mucosa of LPR patients, the characteristic infiltration of neutrophils and lymphocytes caused by exposure to irritants, in the absence of bacteria and spores, already highlighted in previous studies, was observed (4).

RSI, RFS and TNSS

In the treated patients, the RSI, the RFS and the TNSS scores improved significantly at T1 compared with T0 testifying to the improvement in LPR symptoms and signs and nasal symptoms. The RSI and the RFS scores of treated patients worsen at T2, one month after stopping Lmd, compared with T1, but remain significantly lower than at T0 (Table III, Fig. 1A-B). At T0, the RSI and the RFS scores of LPR patients are significantly higher than healthy subjects. Despite the significant improvement in patients treated with Lmd at T1, the difference with healthy subjects remained statistically significant. The median of the TNSS score in the treated patients was the same at T2, one month after stopping Lmd, than at T1 (Table III, Fig. 1C). At T0, the TNSS score in LPR patients is significantly higher than in healthy subjects. Thanks to the significant improvement seen in patients treated with Lmd at T1 and T2, the difference with healthy subjects is statistically less significant.

Rhinocytogram

In treated patients, the presence of lymphocytes is significantly reduced at T1 compared with T0, testifying to the reduction of the inflammatory state. The presence of lymphocytes in treated patients is further reduced at T2, one month after stopping Lmd, compared with T1 (Table IV, Fig. 2A). At T0, the lymphocyte cell count in LPR patients is significantly higher than in healthy subjects. Due to the significant improvement observed in patients treated with Lmd at T1 and T2, the difference with healthy subjects was not statistically significant.

In treated patients, the presence of neutrophils and goblet cells is significantly reduced at T1 compared with T0, indicating a reduction in the inflammatory state. The same cell types in the treated patients were higher at T2, one month after stopping Lmd than at T1, but still significantly better than at T0 (Table IV, Fig. 2B-C). At T0, the neutrophil cell and goblet cell counts in LPR patients are significantly higher than in healthy subjects. Despite the significant improvement in patients treated with Lmd at T1, the differences with healthy subjects remained statistically significant.

Effectiveness of the therapy and adverse effects

After 30 days of therapy (T1), patients treated with Lmd rated the effectiveness of the therapy utilizing an NRS, numbered from 0 to 10. The average rating was 7.13 ± 1.18. One patient treated with Lmd complained of epigastralgia 8 days after starting the product and discontinued treatment.

DISCUSSION

The present clinical study shows a significant improvement in signs, symptoms, and rhino-cytological profile of the nasal mucosa of patients who have LPR and took Lmd for 30 consecutive days. The negative impact of LPR on the respiratory system is mainly caused by inflammation and the accumulation of free radicals in the mucosa, and it is believed that the synergistic action of the ingredients may justify the efficacy of Lmd in treating the signs

Table III. *Medians, 25th and 75th percentiles of the RSI, the RFS and the TNSS scores found in healthy subjects and in patients with LPR at the start of therapy (**T0**), after 30 days of therapy (**T1**) and after 30 days of discontinuation of therapy (**T2**) and statistical significance of comparisons.*

RSI	Healthy subjects (n=27)	Patients treated (n=40)		
		T0	T1	T2
Median [25th – 75th]	2.00 [1.00 – 3.00]	20.00 [17.00 – 23.00]	8.00 [7.00 – 10.00]	10.00 [9.00 – 12.75]
P-value patients treated vs healthy subjects		< 0.0001	< 0.0001	< 0.0001
P-value T1 and T2 vs T0			< 0.0001	< 0.0001
RFS	Healthy subjects (n=27)	Patients treated (n=40)		
		T0	T1	T2
Median [25th – 75th]	0.00 [0.00 – 3.00]	13.00 [12.00 – 14.00]	8.00 [7.00 – 9.00]	9.00 [8.00 – 10.00]
P-value patients treated vs healthy subjects		< 0.0001	< 0.0001	< 0.0001
P-value T1 and T2 vs T0			< 0.0001	< 0.0001
TNSS	Healthy subjects (n=15)	Patients treated (n=22)		
		T0	T1	T2
Median [25th – 75th]	0.00 [0.00 – 2.00]	3.50 [2.00 – 4.25]	2.00 [1.00 – 2.25]	2.00 [1.00 – 3.00]
P-value patients treated vs healthy subjects		0.0002	0.0270	0.0149
P-value T1 and T2 vs T0			< 0.0001	< 0.0001

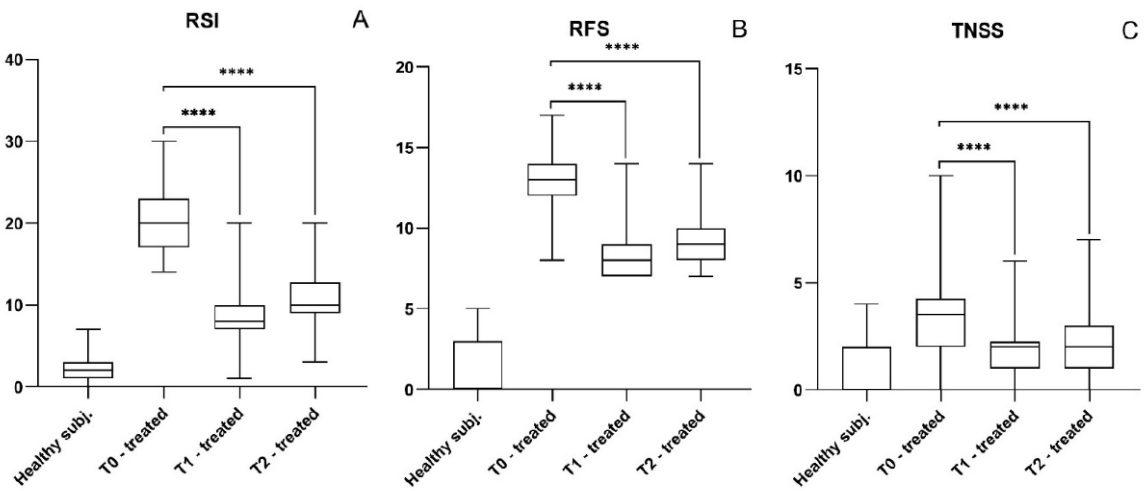

Fig. 1. *Treatment with Lmd for 30 days (**T1**) resulted in a significant reduction in the RSI (**A**), RFS (**B**) and TNSS (**C**) scores which remained significantly lower than the scores at T0 even 30 days after stopping therapy (**T2**) (****p<0.0001).*

and symptoms of LPR. The contact between some ingredients of this formula (alginate, carbonate, and bicarbonate) and the acidic gastric content causes the formation of a 'raft' floating above the gastric content, which impedes its reflux into the oesophagus. In addition, alkalizing substances neutralize the acid pocket formed during a meal. In Lmd, the effects induced by alginate and alkalizing compounds are combined with the therapeutic effects directly induced on the oesophageal and laryngeal mucosa by *O. ficus-indica* cladode extract and *O. europaea* leaf extract. Studies on the therapeutic properties of *O. ficus-indica* have demonstrated an antiulcerogenic activity, which is hypothesized to be determined by the mucilages that form a protective layer on the mucous membranes (9). Studies have shown that aqueous extracts of *O. europaea* can reduce the level of TNF-α in an experimental *in vivo* mouse model and an experimental *in vitro* model on a human lymphocyte cell line. It was also observed that the administration of *O. europaea* extract could prevent the formation of stress-induced gastric lesions in an *in vivo* experimental model. This effect is associated with a decrease in the level of malondialdehyde (an index of lipid peroxidation) and a reduction in the fall in catalase and superoxide dismutase enzyme activity (10). *O. europaea* extract has antioxidant properties, with a protective effect on the mucous membranes. Furthermore, *O. europaea* has been observed to be a stable source of bioactive flavonoids (11). These compounds contribute to the beneficial effects of *O. europaea* extract observed in LPR treatment. Lastly, the protective effect on mucosal cells of the *O. ficus-indica* cladode extract and the *O. europaea* leaf extract has already been shown in vitro in models simulating in vivo conditions (12).

In this study, there was an improvement in all parameters assessed after 30 consecutive days of

Table IV. *Median lymphocyte, neutrophil and goblet cell counts in healthy subjects and LPR patients at the start of therapy (**T0**), after 30 days of therapy (**T1**) and 30 days after discontinuation of therapy (**T2**) and statistical significance of comparisons.*

Cell type	Parameters	Healthy subjects (n=27)	Patients treated (n=40)		
			T0	T1	T2
Lymphocytes	Median [25th – 75th]	0.00 [0.00 – 0.00]	0.50 [0.00 – 2.00]	0.00 [0.00 – 1.00]	0.00 [0.00 – 0.00]
	P-value patients treated vs healthy subjects		0.0029	0.3937	> 0.9999
	P-value T1 and T2 vs T0			< 0.0001	< 0.0001
Neutrophils	Median [25th – 75th]	0.00 [0.00 – 1.00]	2.50 [2.00 – 3.00]	1.00 [0.00 – 1.75]	1.00 [0.00 – 2.00]
	P-value patients treated vs healthy subjects		< 0.0001	0.0164	0.0023
	P-value T1 and T2 vs T0			< 0.0001	< 0.0001
Goblet cells	Median [25th – 75th]	1.00 [1.00 – 1.00]	2.50 [2.00 – 3.00]	1.50 [1.00 – 2.00]	2.00 [1.00 – 2.00]
	P-value patients treated vs healthy subjects		< 0.0001	< 0.0001	< 0.0001
	P-value T1 and T2 vs T0			< 0.0001	< 0.0001

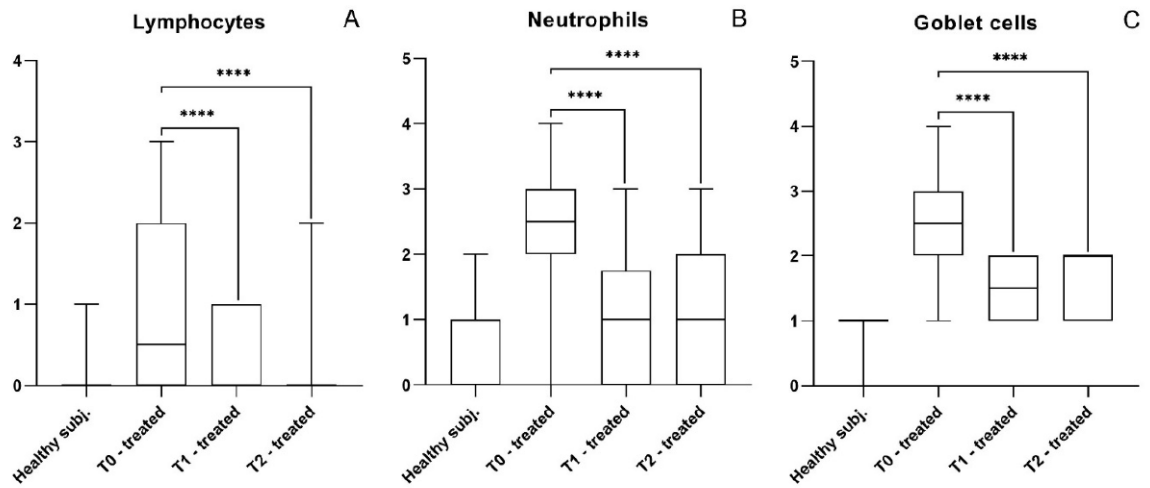

Fig. 2. *Treatment with Lmd for 30 days (**T1**) resulted in a significant reduction, compared with T0, in the number of lymphocytes (**A**) detected, which further reduced 30 days after stopping therapy (**T2**) (******p<0.0001**). At T1 and T2, the number of lymphocytes found in LPR patients was not significantly different from that found in healthy subjects. Treatment with Lmd for 30 days (**T1**) resulted in a significant reduction in the number of neutrophils (**B**) and goblet cells (**C**) detected, which rose slightly 30 days after stopping therapy (**T2**), remaining significantly lower than at T0 (******p<0.0001**).*

taking Lmd by LPR patients. Indeed, the symptoms and signs of LPR, as assessed by the RSI and RFS indices, respectively, improved significantly with treatment.

Cytologically, the element most commonly found in LPR and often associated with nasal symptoms is the neutrophil granulocyte. In addition, there is a percentage increase in goblet cells and an increase in lymphocytes. The rhinocytogram performed at T0 T1 showed that the treatment resulted in a significant reduction in neutrophils, goblet cells and lymphocytes. The reduction of the overexposed elements at the cytological level is accompanied by the already mentioned global improvement of the symptoms, further confirmed by the significant reduction in the TNSS score.

The same parameters, except for the number of lymphocytes, deteriorated slightly in the 30 days following the discontinuation of therapy, showing that in the case of LPR, in order to give stability to the results obtained, it is preferable to continue therapy with the tested medical device for more than one month. Nevertheless, the tolerability of the medical device was good, and the results were considered more than satisfactory by the treated patients.

The results of this clinical study, therefore, demonstrate the efficacy of a medical device composed of magnesium alginate, calcium carbonate, potassium bicarbonate and extracts of O. *ficus-indica* and *O. europaea* in reducing the signs and symptoms commonly associated with LPR and improving the cytological status of the mucous membranes of the upper respiratory tract in patients suffering from this disorder. The study also shows that the medical device used is safe and well-tolerated.

Conflict of interest statement

Enrico Maffezzoni, Federico Maffezzoni, Ketty Luciano and Mario Notargiacomo declare that they have no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article. Stefano Agostini is an employee of Pharma Line.

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REFERENCES

1. Koufman JA, Aviv JE, Casiano RR, Shaw GY. Laryngopharyngeal reflux: Position statement of the committee on speech, voice, and swallowing disorders of the American Academy of Otolaryngology-Head and Neck Surgery. Otolaryngol Head Neck Surg 2002; 127(1):32-5.
2. Kuo CL. Laryngopharyngeal Reflux: An Update. Arch Otolaryngol Head Neck Surg 2019; 3(1):1.
3. Finocchio E, Locatelli F, Sanna F, et al. Gastritis and gastroesophageal reflux disease are strongly associated with non-allergic nasal disorders. BMC Pulm Med 2021; 21(1):53.
4. Mandolesi D, Schiavon P, Ioannou A, et al. Chronic non-allergic rhinitis with neutrophils is associated with higher acid exposure time: a pH-impedance monitoring study. Dig Liver Dis 2020; 52(4):414–9.
5. Dagli E, Yüksel A, Kaya M, et al. Association of Oral Antireflux Medication with Laryngopharyngeal Reflux and Nasal Resistance. JAMA Otolaryngol Head Neck Surg 2017; 143(5):478-83.
6. Lechien JR, Saussez S, Schindler A, et al. Clinical outcomes of laryngopharyngeal reflux treatment: A systematic review and meta-analysis. Laryngoscope 2019; 129(5):1174-87.
7. Gelardi M, Iannuzzi L, Quaranta N, et al. Nasal cytology: practical aspects and clinical relevance. Clin Exp Allergy 2016; 46(6):785-92.
8. Gelardi M. Atlante di Citologia Nasale. Edi Ermes, Milano, 2012.
9. Galati EM, Mondello MR, Giuffrida D, et al. Chemical characterization and biological effects of Sicilian Opuntia ficus indica (L.) Mill. Fruit juice: antioxidant and antiulcerogenic activity. J Agric Food Chem 2003; 51(17):4903-08.
10. Dekanski D, Janićijević-Hudomal S, Ristić S, et al. Attenuation of cold restraint stress-induced gastric lesions by an olive leaf extract. Gen Physiol Biophys 2009; 28 Spec No:135-42.
11. Goulas V, Papoti VT, Exarchou V, et al. Contribution of flavonoids to the overall radical scavenging activity of olive (Olea europaea L.) leaf polar extracts. J Agric Food Chem 2010; 58(6):3303-08.
12. Rizza L, Frasca G, Nicholls M, et al. Caco-2 cell line as a model to evaluate mucoprotective proprieties. Int J Pharm 2012; 422(1-2):318-22.