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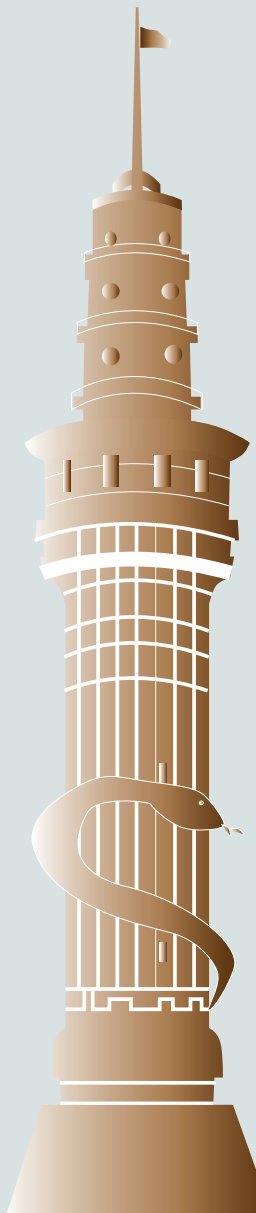

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The development and *in vitro* evaluation of benzydamine hydrochloride medicated chewing gum formulations

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ABSTRACT

The objective of this study was to develop a chewing gum formulation for benzydamine hydrochloride (BN) and evaluate the effect of different co-compression agents on physicochemical parameters and *in vitro* drug release profile. BN has been utilized in symptomatic treatment for pain and irritations of the mouth and throat. Chewing gum formulations of BN were produced using direct compression method. In fabricated chewing gums, except pharmacopeia analysis, physical and structural analysis like thickness, weight variation, elasticity and compressibility were evaluated. The release of BN from the gum was studied using the chewing apparatus which have been designed for procuring the release of BN from the chewing gum.The quantity of BN present in the chewing gum and the release medium were quantified by spectrophotometric method. Gum formulations showed promising *in vitro* release profiles, in which 80–90% BN was released in a sustained manner over 20 min of chewing time. In addition, it was observed that the drug release was fitted into matrix diffusion kinetic and revealed a non-Fickian drug release mechanism. This study suggests that BN in a gum formulation is a suitable dosage form for the delivery in the oral cavity, thereby and serving as an instant analgesic.

Keywords: Benzydamine hydrochloride, medicated chewing gum, mastication apparatus

INTRODUCTION

Benzydamine hydrochloride (BN), a tertiary amine derivative, is a non-steroidal anti-inflammatory agent that possesses analgesic, anesthetic, anti-inflammatory, anti-pyretic and anti-microbial properties. However its oral, topical and oro-mucosal dosage forms are currently available in the market, it is commonly in use for the relief of inflammatory conditions of the oral cavity soft tissues and skin.

In a mouth-rinse formulation as a concentration of 0.15 % BN, its use has been indicated in the cure for recurring oral disorders as aphthous stomatitis, burning mouth syndrome, sore throat and radiation-induced oral mucositis (Herrera 2005).

Since the ancient times, chewing gum has been used world-wide, however the concept of using chewing gum as a drug delivery system has been implemented for more than 100 years. *Aspergum*, which contains acetyl salicylic acid, is the first medical chewing gum (Noehr-Jensen et al. 2006). It was marketed in 1924 in the USA and is still in the market. Chewing gums gained broader attention as a drug delivery system with the introduction of nicotine containing chewing gums into the market in 1978 (Rowe 2003). Twenty years later, in 1998, drug containing chewing gums were included in the European Pharmacopoeia with the name: '*medicated chewing gum*' (European Pharmacopoeia 9th edition).

The definition of medicated chewing gum in the European Pharmacopoeia is stated as; "a single-dose, solid preparation with tasteless masticatory gum base, mainly consisting of gum which is intended to be chewed and not swallowed, providing a slow steady release of the medicine contained", and it is intended to be used as "local treatment of mouth diseases or systemic delivery after absorption through the buccal mucosa or from the gastrointestinal tract" (Herrera 2005). Medical chewing gums have

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been formulated with different kinds of drugs beside nicotine (Cherukuri et al. 2000; Morjaria et al. 2009) and aspirin (Woodford and Lesko 1981) such as nystatin (Andersen et al. 1990), miconazole (Pedersen and Rassing 1990), caffeine (Tyrpin et al. 2002), antacids (Zyck et al. 2003), anti-microbial decapeptide (Dong et al. 2005), ondansetron hydrochloride (Nagaich et al. 2010), cetirizine (Stojanov et al. 2012; Swamy et al. 2012), dextromethorphan hydrobromide (Swamy et al. 2012), dimenhydrinate hydrochloride (Mehta and Trivedi 2011).

Medicated chewing gums have so many benefits compared to other conventional oral mucosal dosage forms that are intended to be used for local treatment of mouth diseases like gingivits and dry mouth sydrome (Maggi et al. 2005). Chewing gums are also useful for providing tooth hygiene and caries-prevention by activating the masseter muscles and stimulating secretion of saliva that contains rich bicarbonate ions, in which increase in plaque pH reduce the risk of caries formation (Abelson et al. 1990; Dodds et al. 1991). Chewing gum can be retained in oral cavity for long times and with the help of the saliva circulation within the mouth, drug could be effectively distributed inside the whole mouth. As a dosage form, gum could be taken easily and discretely without any need of water intake and also in any case prompt discontinuation of medication is possible. Besides, the use of chewing gum as a drug delivery system can also improve patient acceptance and compliance. In addition to offering clinical benefits, medicated chewing gums have also good physical and chemical stability during the use and the storage as well (Maggi et al. 2005).

Chewing gum consists basically of a neutral and tasteless masticatory gum-base and several non-masticatory ingredients, such as fillers, softeners, sweeteners, flavouring and texture regulating agents (Rassing 1992; Eisenstadt et al. 1998). All excipients in the gum formulation gives different properties to the formulation like anti-sticking effect to the teeth, long lasting flavor and improved texture. After non-sugar gums gain attention, combinations of sorbitol, xylitol, mannitol, aspartame and acesulfame potassium is started to use in sugar free gums and medicated gums (Maggi et al. 2005).

There are three main methods given in the literature which are properly used for production of medicated chewing gums named as; conventional and direct compression method and ion exchange method which is specialized for nicotine containing chewing gum. Among these three methods, direct compression which is developed in compliance with the conventional tablet compression technology brought advantages as; the easiness of production steps, cost and time effectiveness, by using low temperatures allows us to use sensitive bioactive and phytochemical components by this way it can prevent the potential stability problems, low moisture prolong the shelf life of the active in the gum and higher dosage of active could be formulated within gum. However, the main drawback of these methods is the sticking effect of the gum base to the compression punches of the tableting equipment. This problem originates from the adhesive nature of the gum base, major component of the formulation; due to this reason, production speed should be held at slow and cooling procedure could be applied optionally. To prevent any sticking problems, whole tableting machines and their tools should be kept at temperatures below 18°C during the production process. And it should be noted that the temperature should not be as low to interfere the production procedure of medicated chewing gums therefore the temperature should be held above 10-12°C (Testa 1999; Mostafavi et al. 2014).

The recommended solution to overcome the sticking problem is using co-compression agents as glidants and lubricants in different manners but in some formulations it causes organoleptic problems and it could change the release profile of the drug from the chewing gum so it is crucial to find the exact amount for each ingredient in the formulation receipt.

The primary objective of this work was to develop a chewing gum formulation for BN and investigate the influence of different lubricants on physicochemical parameters and drug release profile.

MATERIALS AND METHODS

Materials

Benzydamine hydrochloride was a gift sample, provided by Santa Farma (Istanbul, Turkey). Health in Gum, compressible gum base powder mixture was obtained from Cafosa Gum S.A. (Barcelona, Spain) as a sample gift, Colloidal silicon dioxide from Evonik Industries (Germany), and the other chemicals provided as a gift sample from Roquette (France). All the other compenents are in the analytical grade.

Methods

Chewing gum mastication apparatus

A chewing gum mastication apparatus was designed to simulate the chewing behaviour for the release of benzydamine from the gums. The designated apparatus was used to evaluate the releasing pattern of the drug from gum. Briefly, the fully stainless steel wall of the vessel was used to be able to keep the temparature equal inside the whole vessel during the mastication of gum samples.

The instrumental settings were adjustable for temperature and mastication rate. In the present experiment the following settings and conditions were used. Temperature of the test medium was set at 37°C; 10 ml of artificial saliva buffer, pH

Table 1. Composition of artificial saliva*

Composition	Amount (g/L)
Potassium chloride	0.720
Sodium chloride	0.600
Calcium chloride dihydrate	0.220
Citric acid	0.030
Potassium thiocyanate	0.060
Potassium bicarbonate	1.500
Potassium phosphate monobasic	0.680
Sodium phosphate dibasic	0.886

* Swamy et al. 2012

6.75, (Table 1) was used as the volume of the test medium. 50 strokes per minute was implemented for chewing frequency. The distance between the upper and lower jaws set was at 1.6 mm and total chewing time was 60 min. Aliquots of 1 ml were withdrawn from each time point from the chamber at 0, 5, 10, 15, 30 and 60 min during the *in vitro* releasing procedure. The samples were diluted for analyzing the drug concentrations in the test medium (Kvist et al. 2000).

Spectrophotometric analysis

Spectrophotometric measurements of BN samples were carried out using the UV-1601 model UV-VIS spectrophotometer with 1 cm quartz cell. A standard stock solution of BN reference standard (100 µg/mL) was prepared in a 10 mL calibrated flask in artificial buffer (pH 6.75). A validation set, consisting of six solutions in working range of 1-60 µg/mL, was freshly prepared and scanned in UV region. This process was repeated three times for each concentration. The absorption maxima, observed at 308 nm, was recorded and plotted against concentration, which followed the Beer and Lambert's law and gave a straight line (R^2 =0.9998).

Preparation of Benzydamine Hydrochloride chewing gum

Direct compression method was used to produce twenty seven chewing gum powder formulations containing BN. Formulation of chewing gum mainly consisted of gum base powder, fillers, active ingredients (drug), and flavoring agents as shown in Table 2. At the fist stage of production steps, BN was mixed with aspartame- acesulfame potassium (1:2 ratio) as a suitable sweetener by using cubic shaker for 15 min.Then this mixture was directly mixed with compressible gum base powder for another 10 min. Since BN has slightly bitter taste, menthol flavor (0.5 %) was used to mask its bitterness and unpleasant taste. Gum powder mixture which was obtained by adding co-compression agents to previous powder mixture, became ready for compression. Gums were compressed in oval shapes, in every piece of which 2.3 g of powder was weighed by using Korch EK-0 model hydraulic tablet compression machine. To prevent probable sticking problems, not only whole tableting machines and their tools were kept at temperatures below 18°C, but also co-compression agents as glidants; colloidal silicon dioxide and lubricants; magnesium stearat in different manners were used during the production process.

Determination of pre-compression parameters

The formulated chewing gum granules for each formulation in Table 2 were evaluated for pre-compression parameters such as bulk Density (*Vb*), tapped density (*Vt*), Hausner's ratio (*H*) and Carr's Index (*I*) which are indicative parameters for flow and compressibility (European pharmacopoeia, 9th edition).

$$Bulk\ Density(Vb) = \frac{Mass}{Bulk\ Volume}$$

$$Tapped\ Density\ (Vt) = \frac{Mass}{Tapped\ Volume}$$

$$Hausner's\ Ratio\ (H) = \frac{Vt}{Vb}$$

$$Carr's\ Index\ (I) = \frac{Vt - Vb}{Vt} * 100$$

Table 2. Selection and optimization of excipient in Benzydamine Hydrochloride chewing gum formulation

Formulation Code	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18	F19	F20	F21	F22	F23	F24	F25	F26	F27
Gum Base Composition (HiG PWD-01) (%)	93	93	93	93	93	93	93	93	93	95	95	95	95	95	95	95	95	95	97	97	97	97	97	97	97	97	97
Benzydamine Hydrochloride (%)	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Magnesium Stearat (%)	0.25	0.5	0.75	0.25	0.5	0.75	0.25	0.5	0.75	0.25	0.5	0.75	0.25	0.5	0.75	0.25	0.5	0.75	0.25	0.5	0.75	0.25	0.5	0.75	0.25	0.5	0.75
Aerosil 200® (%)	0.5	0.5	0.5	1	1	1	1.5	1.5	1.5	0.5	0.5	0.5	1	1	1	1.5	1.5	1.5	0.5	0.5	0.5	1	1	1	1.5	1.5	1.5
Aspartame-Asesulfam K (1:2,%)	5.25	5.0	4.75	4.75	4.25	4.25	4.25	4.0	3.75	3.25	3.0	2.75	2.75	2.5	2.25	2.25	2.0	1.75	1.25	1.0	0.75	0.75	0.5	0.25	0.25	0	0
Menthol (%)	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87

Determination of post-compression parameters
The chewing gum tablets, shown in Table 2, were subjected to weight variation test ast uniformity of mass and drug content, tablet breaking force/hardness and friability determination.

Uniformity of mass
Uniformity of mass is mainly implemented for non-coated compressed dosage forms. Ten unit of gums were taken randomly from different gum formulations and weighed individually. The arithmetic mean weight was used to calculate the results. The dosage form formulation comply with the test; on condition that, not more than two of the individual masses deviate more than 5%, from the average mass (European pharmacopoeia, 9th edition).

Uniformity of drug content
Ten units of chewing gum from randomly selected formulations from Table 2 were used to determine drug content in prepared chewing gums. Each gum piece was crushed separately and transferred into a 10 mL volumetric flask and 10 mL artificial saliva was added. After 1 h mixing with magnetic stirrer, 1 mL of sample was taken and diluted with artificial saliva to 10 mL. The absorbance of solution was measured spectrophotometrically at 308 nm. The standard curve was used to calculate BN concentration. The formulation complies with the pharmacopeial test limits, only if the individual drug content result is between 85% and 115% of the average content (European pharmacopoeia, 9th edition).

Friability
To evaluate friability of compresed gums, ten units of gum formulations from the twenty seven formulations were randomly chosen and attentively de-dusted prior to test. Then gums were accurately weighed and placed in the drum of a Sotax® HT-1 Friabilator. The drum was rotated 100 times at 25 rpm, and then gums were removed from the drum. Loose dust was removed from the gums as mentioned above and reweighed accurately. The difference in two weights represents the friability of formulation. A maximum loss of mass (obtained from a single test or from the mean of three tests) not greater than 1.0% is considered admissible (European pharmacopoeia, 9th edition).

***In vitro* drug release from chewing gum**
The standardized equipment for disintegration, dissolution and drug release testing, used for the conventional oral dosage forms could not be considered as appropriate for the medicated chewing gums because the drug release process from medicated chewing gum is quite different compared to other conventional oral dosage forms fundamentally. For evaluation of the drug release from chewing gum, both of the dosage form and the chewing activity would influence the drug release which should be considered in release studies. Furthermore, gums are not intended to dissolve/disintegrate by themselves so a mechanical force inside the mouth is needed to cause the active release drug from the chewing gum (Liljewall 1992). Therefore,the European Pharmacopoeia guidelines recommend to use of a spesific device for *in vitro* release studies from medicated gum formulations which is able to simulate the human chewing behavior (European pharmacopoeia, 9th edition). But high cost of the equipment prompt most of the research groups to find alternative solutions as designing new equipment which can fulfil researchers needs (Gajendran et al. 2008).

In the current study, all twenty seven BN chewing gums have been tested by using the mastication apparatus. Artificial saliva (10 mL) was placed to mouth stimulating part of mastication apparatus and a prepared chewing gum was placed on its predesigned place in the apparatus. The ragged piston, designed to simulate the teeth, was adjusted. The mechanical mixer (as a motor) was attuned on 50 rounds per minute. The masticating apparatus was started to release the drug from the chewing gum by mechanical forces.

Aliquots of 1 ml were withdrawn from each sample at 5, 10, 15, 20, 30, 40, 50 and 60 min. during the releasing test and diluted to 10 mL for spectrophotometric analysis. The medium was replaced by an artificial saliva (pH 6.75 at 37°C) after each sampling. The experiments were replicated with placebo chewing gums as well. The percentage of drug released during the mastication process was calculated by subtracting the absorbance of the active ingredient, present in the gum from the absorbance of the placebo gum.

***In vitro* drug release kinetics**
For understanding the mechanism of drug release and release rate kinetics of the drug from the dosage form, the obtained data was fitted into the equations belongs to zero order, first order, Higuchi matrix, Krosmeyer & Peppas model by analyzing the values, the best fit model was selected (Peppas 1985; Swamy et al. 2010)

Influence of co-compression agents on *in vitro* drug release profile
In this study, beside keeping the environmental temperature of production below 18°C, various amounts of co-compression agents were also employed to overcome the sticking problem during the production of the chewing gums. While using co-compression agents in the production, their effect on release profiles should be taken into consideration. Therefore, quantity bound effect of these agents on the release profiles within the study evaluated.

Statistical analysis
All testing is performed in accordance with the pharmacopoeia requirements. In any cases in which the statistical analyses were required, the paired t-test was performed. A significant difference was $p < 0.05$ used.

RESULTS

Gum formulations were visually inspected and physically evaluated for appearance, color, stickiness, and plasticity, which seem suitable. All gums were prepared in an oval-shape with 2 cm diameter and 1 cm thickness and the prepared gums had smooth and soft surface.

The physical parameters of the granules for all the formulated batches exhibited good flow properties, which is indicated by the bulk and tapped density in the range of 0.465 g/mL to 0.575 g/mL and 0.540 g/mL to 0.610 g/mL respectively.

The Carr's index was found to be in the range of 5.263 % to 9.615 % and Hausner's ratio was in the range of 1.044 -1.093, which is found to be acceptable.

The mean weight of 20 chewing gum tablets from each twenty-seven formulation were determined. None of the tablets deviated by more than 5% from the mean weight, indicating that all the formulations fulfilled the pharmacopeial limits for weight variation.

The hardness of the compressed chewing gum formulation was found to be in range of 9.8 to 11.7 kg/cm^2. All the formulations were found to conform to pharmacopeial limits of the label claim.

Randomly selected medicated chewing gum formulations passed tests for uniformity of mass with an average mass of 2301.4 mg and non of the formulations were deviated from ±5% of average mass of chewing gums.

All 10 units of medicated chewing gums from twenty seven formulations, which were sampled randomly, have shown suitable uniformity of content results as contents of BN in all 10 gum formulations have fallen within a compliance limit of 85–115% and the average content of BN was found to be 2.98 mg ± 0.34%.

In friability test, after 100 rotations, the total weight loss of 10 units of medicated chewing gums were found to be 0.36% which was less than the compliance limit of 1.0 %; so selected gum formulations have passed in the friability test.

As the instrumental settings, the chewing frequency, temperature of the test medium, distance between the upper and lower jaws, and chewing times are four factors which may affect the drug release from the gum base, optimised prior to release test.

Chewing gum formulations containing BN as active substance were prepared with different ratio of compressible gum base powder, sweetening and co-compressing agents. Within this study, we mainly evaluated the effect of the compressible gum base powder co-compression agents' ratio on *in vitro* release profile. The rate and amount of drug released from the gum formulations was determined in artificial saliva (pH 6.75), due to the pH of the saliva is between 6.3 -7.2. Drug release was tested for all formulations that is shown in Table 2 and the mean releasing percentage and the related standard deviations are shown in Figures 1-3.

The release profile obtained after chewing are a proof of the efficiency of this dosage form, as the BN content in the residual gum decreased by increasing the mastication time for all the formulations. Contrary to the expectations, while formulation F7 which is produced with lower amounts of gum base powder and higher amount of aspartame-acesulfame and colloidal silicon dioxide, showed slower release profile; formulation F20 which is prepared with higher amount of gum base powder, showed faster release and released the 65% of the BN within 5 min. and 96,5 % in 20 min. However, formulation F22 that is prepared with lowest amount of lubricant and high amount of glidant showed the gradually increasing release profile. After 5 min was about 15% and after 20 min, the release of the BN was reached about 93%. This finding may propose a longer oral presence of BN in oral cavity as a chewing gum formulation.

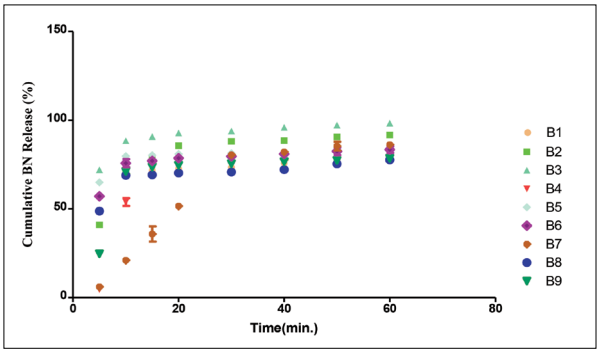

Figure 1. *In vitro* release profile of 93% gum base powder containing gum formulations with different ratio of magnesium stearate vs aerosil 200 (Mean SD, n=3)

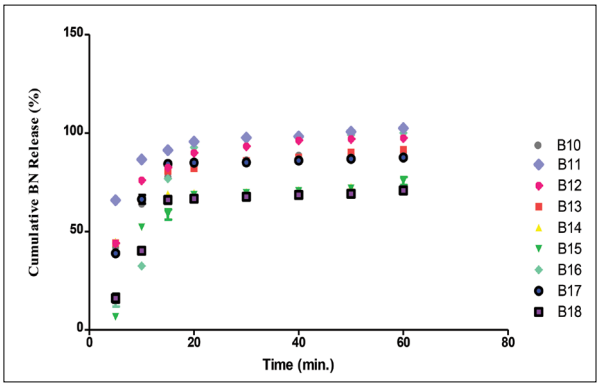

Figure 2. *In vitro* release profile of 95% gum base powder containing gum formulations with different ratio of magnesium stearate vs aerosil 200 (Mean SD, n=3)

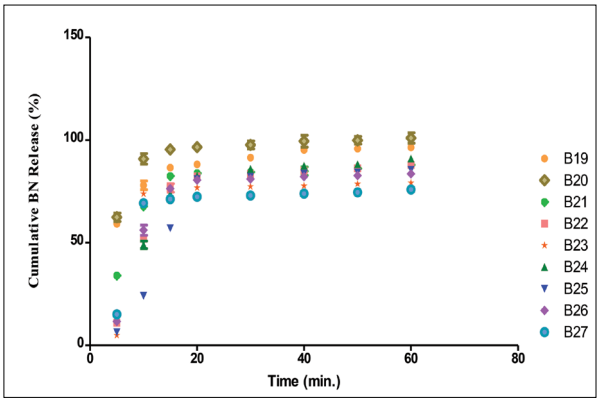

Figure 3. *In vitro* release profile of 97% gum base powder containing gum formulations with different ratio of magnesium stearate vs aerosil 200 (Mean SD, n=3)

In all formulations, the determination coefficiency (r^2) values of Korsmeyer Peppas model were close to 1. The diffusion coefficients (n) values ranged from 0.5113 to 0.9988. Since the r^2 values of Korsmeyer Peppas matrix were close to 1, the drug release follows matrix diffusion kinetics. Hence it was concluded that diffusion was the mechanism of the drug release from the medicated chewing gums. Further, observed diffusion coefficient values are indicative of the fact that the drug release from the formulation follows non-Fickian transport mechanism.

Table 3. In vitro Release Kinetics **

Mathematical models	Equation
Zero order	$F=k_0t$
First order	$Ln(1-F)=-k_t t$
Higuchi	$F=k_h t^{1/2}$
Korsmeyer-Peppas	$F=k_K\text{-}_P t^n$

*F denotes fraction of drug released up to time t. k_0, k_t, k_H, $k_{1/3}$, $k_{K\text{-}P}$ are constant of the mathematical models. n is the release exponent for the Korsmeyer-Peppas model **(Peppas 1985)

These results provide a proof that this dosage form is a good administration system which is able to guarantee a fast and complete drug release after a reasonable chewing time.

DISCUSSION

At first glance, chewing gums are mainly considered as a confectionery product. However, after medicated chewing gums had become available in the pharmaceutical market, chewing gum paved the way for a more general acceptance as a drug delivery system (Mostafavi et al. 2014). Nowadays, this new drug delivery system has established itself on the market and achieved a reasonable acceptance by both professionals and patients (Rathbone et al. 2002; Rassing et al. 2003). Beside this, when local effect is targetted, chewing gums gain more attention as a drug delivery tool.

Various formulations should be prepared and tested to provide a chewing gum with acceptable organoleptic and technological properties. A pleasant taste is a prerequisite for this dosage form. An optimal chewing volume, a long-lasting taste, anti-adherent properties to the teeth, and acceptable pharmaceutical properties such as fast and complete drug release from the prepared formulation must be considered as well (Maggi et al. 2005).

Drug delivery from a medicated chewing gum has completely different dynamics compared to conventional oro-mucosal drug delivery systems. While assesing drug release from chewing gum, chewing activity of the patient should be considered as one of the main factors that has a great influence on drug release. Releasing of the active substance from gum formulation is not performed by disintegrating and/or dissolving by the gum itself, therefore a mechanical treatment of the dosage form is essential.

It is observed from the study that, drug release from the gum to saliva is affected from mechanical forces, temperature, and water permeation. In fact, under sink conditions, the rate of drug release is directly related to the chewing frequency and solubility of drug in buccal cavity and is indirectly related to the mass of the gum base (European Pharmacopoeia, 9th edition). Thus, a specific mastication device was employed for performing drug release to simulate human chewing behavior. However, in some formulations, the co-compression agents caused sandy effect and reduced the chewibility of gum formulations and ratio between the gum base: glidant: lubricant has a great influence on *in vitro* release profiles of the gum formulations therefore the affect of anti-adherence agents on release profile assesed.

Consequently, F22 formulation which is consist of %97 gum base, % 13 BN, %0,5 magnesium stearate, %1 Aerosil, %.0.75 Aspartame-Asesulfam K (1:2) and %87 menthol, was selected due to its *in vitro* release profile and *in vitro* release characteristics.

CONCLUSION

The study accomplishes the probability of the formulation of the directly compressible chewing gum of BN using Health in Gum (gum base powder) with the improved taste and compressibility by using combination of the sweeteners and co-compression agents. It will give quick analgesic effect. Moreover, it can be taken anywhere anytime without preventing patient from living an active life which promotes very high patient acceptance and higher patient compliance.

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Microbiological burden of public transport vehicles

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ABSTRACT

The goal of this study was to determine the role of public transport in the transmission of microorganisms. A total of 60 swab samples were collected in the morning and evening from handles in public transport trams, metrobuses, and buses. Swab samples were examined using microbiological methods, and the number and types of microorganisms were determined. Total aerobic bacterial and fungal counts in samples collected in the evening were higher than those in samples collected in the morning from trams and metrobuses. However, the total bacterial and fungal counts were very high in samples collected in the morning and evening from buses. Staphylococcus aureus, coagulase-negative staphylococcus, and Enterococcus spp. were isolated from these samples. The results of our study show that public transportation can be a significant reservoir for spreading pathogenic microorganisms. For this reason, it is very important to regularly follow cleaning and hygiene rules and to inspect these vehicles.

Keywords: Microbiological burden, microbial contamination, public transport

INTRODUCTION

As part of living in society, many common spaces are shared with other people. This makes it possible to spread diverse microorganisms that can lead to infections. People who use public transport can pass bacteriological, virological, or fungal infections to other people (Rusin et al. 2002). The greatest risk for infectious diseases in these vehicles is that people sit close together in a closed environment and breathe the same air (Yatağan 1991; Furuya 2007; Edelson and Phypers 2011). These vehicles can become a significant source of microorganisms when passengers do not close their mouths when coughing and sneezing. Handles, seats, anchors, floors, and windows are areas that can host infectious microorganisms.

For this reason, detailed internal cleaning of public transportation vehicles, which thousands of people use every day, is an important issue. In İstanbul, public transport services are mainly run by İstanbul Electricity Tramway and Tunnel Businesses (İETT). According to IETT's official website, the institution serves in İstanbul with 4558 public and private buses and 334 metrobuses. The number of buses is about 5000, which means there are 3,440,000 passengers every day. İETT buses and other vehicles are reported to be cleaned to provide healthier environment for passengers (İETT 2015).

In our study, the microbiological burden of vehicles was evaluated in order to determine the role of public transportation vehicles in the transmission of microorganisms that can cause significant infections in humans.

MATERIALS AND METHODS

Sample collection

In this study, we investigated the microbiological burden of public transport such as trams, metrobuses, and buses, which are frequently used in daily life. A total of 60 samples were taken from the handles of these vehicles. Samples were taken from vehicles by morning and evening by swap method.

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Swab method

The samples were taken with swap, which is the popular testing method. In a 10x10 cm area, sampling was. Swaps were placed in a tube containing Amies agar gel transport media and brought to the laboratory for analysis.

Microbiological analysis

All the collected swap samples were analyzed to detect the presence of total bacterial and fungal count. The microbial species *S. aureus, Pseudomonas aeruginosa, Escherichia coli*, Salmonella sp. and Enterococcus sp. were investigated, as suggested in The United States Pharmacopeia (USP). Each sample was weighed aseptically and diluted in pH 7 phosphate buffer and Tryptic Soy Broth (Difco). Serial dilutions of the samples were homogenized in phosphate buffer and spread on duplicate Tryptic Soy Agar (Difco) and Sabouraud Dextrose Agar (Difco). Plates were incubated at 37°C 48h and 25°C 5-7 days. At the end of the incubation, emergent colonies were counted and the numbers of colony forming units (CFU/ml) were determined. Tryptic Soy Broth was incubated at 37° C 24h. After incubation, samples were spread on Mannitol Salt Agar (Oxoid), Baird-Parker Agar (Difco), Cetrimide Agar (Difco), MacConkey Agar (Difco), Eozine Methylene Blue Agar (Difco), Fluid Tetrathionate Medium (Difco), Xylose-Lysine Deoxycholate Agar (Difco), Bismuth Sulfite Agar (Difco) and Enterococcosel Agar (Difco) to determine the presence of specific microorganisms according to pharmacopeia (USP 2009). Plates were incubated at 37°C 24h and identification was performed after microscopic examination and biochemical identification.

RESULTS

Microbiological analysis

Sixty samples taken from the handles belonging to 10 different trams, metrobuses and buses in the morning and evening were microbiologically examined.

Determination of total aerobic bacteria and fungus numbers in the samples belonging to the trams and the isolated microorganisms

A total number of bacteria and fungi counts and isolates determined in the samples belonging to the trams in the morning and evening were summarized in Tables 1 and 2. The total number of bacteria for one of the samples taken in the morning, the total number of bacteria in the eight samples taken in the evening and the total number of fungi in one sample were found high.

Table 1. The total aerobic bacteria, fungi counts and isolated microorganisms in the samples taken from the trams in the morning

Sample Area	Sample No	Total Bacterial Count (cfu/mL)	Total Fungal Count (cfu/mL)	Isolated Microorganisms
	1	-	-	-
	2	$2x10^1$	$3x10^1$	KNS
	3	$<1x10^1$	-	*Enterococcus spp*, KNS
	4	-	-	KNS
Handles	5	$1x10^1$	-	S.aureus
	6	-	-	KNS
	7	$6.28 x10^5$	-	-
	8	-	$2x10^1$	-
	9	$6x10^1$	-	KNS
	10	$5x10^1$	-	*Enterococcus spp*

Table 2. The total aerobic bacteria, fungi counts and isolated microorganisms in the samples taken from the trams in the evening

Sample Area	Sample No	Total Bacterial Count (cfu/mL)	Total Fungal Count (cfu/mL)	Isolated Microorganisms
	1	$2.8 x10^3$	$3x10^1$	-
	2	$3.4x10^2$	-	*S.aureus*
	3	$1.83x10^3$	$1.34x10^3$	*S.aureus*
	4	-	$2.5x10^1$	-
Handles	5	$1.14x10^3$	-	-
	6	$2.2x10^2$	$1x10^1$	-
	7	$2.62 x10^3$	-	*Enterococcus spp*,
	8	-	-	-
	9	$1.25x10^6$	-	-
	10	$3.12x10^5$	$5x10^1$	*S.aureus*

Determination of total aerobic bacteria and fungus numbers in the samples belonging to metrobuses and the isolated microorganisms

A total number of bacteria and fungi counts and isolates determined in the samples belonging to the metrobus in the morning and evening were summarized in Tables 3 and 4. Eight of the samples taken in the morning and seven of the samples taken in the evening were found to have a high total number of bacteria.

Determination of total aerobic bacteria and fungus numbers in the samples belonging to buses and the isolated microorganisms

A total number of bacteria and fungi counts and isolates determined in the samples belonging to the buses in the morning and evening were summarized in Tables 5 and 6. The total number of bacteria in the six samples taken in the morning and the total number of fungi in one sample were found to be high. The total number of bacteria and fungi in the all samples taken in the evening were also high.

DISCUSSION

Public transportation vehicles facilitate the spread of various pathogens that can cause frequent infections in the community. These vehicles are vectors of colds, flu, and bronchitis in winter months. Many public transport vehicles carry passengers well above their capacity, especially in the morning and evening hours. This leads to the spread of disease among people using these vehicles. To ensuring proper hygiene, these vehicles must be inspected regularly.

Hand-touch sites can become contaminated with bacteria and become fomites for the transmission of bacteria between humans. Such sites can provide a reservoir for community-associated bacteria in high-prevalence areas [Brook and Brook (1994); Rusin et al. (2002); Simoes et al. (2011); Zhou and Wang (2013)]. Stepanovic *et al.* (2008) recently reported a high frequency of methicillin-resistant, coagulase-negative staphylococci but no MRSA contaminating hand-touch sites on public transportation vehicles in Serbia (Stepanovic et al. 2008). Otter and French determined total aerobic counts in 118 hand-touch surfaces on buses, trains, stations, hotels, and public areas of a hospital in central London. *S. aureus* isolates were identified (Otter and French 2012).

According to the information provided by the İETT General Directorate, vehicles that serve millions of people every day have a detailed interior and exterior cleaning to provide a more healthy environment for the passengers. It is stated that these

Table 3. The total aerobic bacteria, fungus counts and isolated microorganisms in the samples taken from the metrobuses in the morning

Sample Area	Sample No	Total Bacterial Count (cfu/mL)	Total Fungal Count (cfu/mL)	Isolated Microorganisms
	1	$5.1x10^4$	-	-
	2	$6.25x10^5$	-	KNS
	3	$3.6x10^5$	-	*Enterococcus spp*, KNS
	4	$4.26x10^5$	-	KNS
Handles	5	$3.83x10^5$	-	KNS
	6	$2.6x10^4$	-	KNS
	7	$5.42x10^5$	-	-
	8	$6.25 x10^6$	-	-
	9	4.10^1	-	*S.aureus*
	10	$4.5.10^1$	-	*S.aureus*

Table 4. Total aerobic bacteria, fungus counts and isolated microorganisms in the samples taken from the metrobuses in the evening

Sample Area	Sample No	Total Bacterial Count (cfu/mL)	Total Fungal Count (cfu/mL)	Isolated Microorganisms
	1	$3.22x10^5$	-	KNS
	2	$8.21x10^4$	-	KNS
	3	-	$2x10^1$	KNS
	4	$7.11x10^5$	-	KNS
Handles	5	-	-	KNS
	6	$2.64x10^5$	$2x10^1$	KNS
	7	-	-	KNS
	8	$3.24 x10^5$	-	KNS
	9	$6.14x10^5$	-	KNS
	10	$2.13.10^5$	-	KNS

Table 5. The total aerobic bacteria, fungus counts and isolated microorganisms in the samples taken from the buses in the morning

Sample Area	Sample No	Total Bacterial Count (cfu/mL)	Total Fungal Count (cfu/mL)	Isolated Microorganisms
	1	$2.6x10^2$	-	*S.aureus*
	2	$3x10^1$	-	*Enterococcus spp,*
	3	$1.2x10^2$	-	*Enterococcus spp,* KNS
	4	$1.3x10^2$	-	*Enterococcus spp,*KNS
Handles	5	$1x10^1$	-	*Enterococcus spp,* KNS
	6	$7x10^1$	-	*Enterococcus spp,* KNS
	7	$4x10^1$	-	*Enterococcus spp,* KNS
	8	$1.8 x10^4$	-	*Enterococcus spp,* KNS
	9	$5.6x10^2$	-	*Enterococcus spp,* KNS
	10	$9x10^2$	$1.7x10^3$	*Enterococcus spp,* KNS

Table 6. The Total aerobic bacteria, fungus counts and isolated microorganisms in the samples taken from the buses in the evening

Sample Area	Sample No	Total Bacterial Count (cfu/mL)	Total Fungal Count (cfu/mL)	Isolated Microorganisms
	1	$3.83x10^5$	$2.6x10^2$	*S.aureus*
	2	$3.14x10^5$	$3.2x10^2$	*S.aureus*
	3	$6.81x10^4$	$1.3x10^2$	*Enterococcus spp, S.aureus*
	4	$7.11x10^5$	$1.5x10^2$	*S.aureus*
Handles	5	$1.24x10^4$	$1.4x10^2$	*S.aureus*
	6	$2.4x10^6$	$3.25x10^4$	*Enterococcus spp, S.aureus*
	7	$1.3x10^4$	$1.09x10^2$	*S.aureus*
	8	$7.1x10^5$	$6.1x10^5$	*S.aureus*
	9	$3.25x10^4$	$1.37x10^4$	*S.aureus*
	10	$1.71x10^5$	$1.59x10^2$	*S.aureus*

routine cleanings are carried out every day by 4:00 am as the vehicles are readied for morning service. It is also stated that the vehicles are subjected to detailed disinfection treatment once a week, thus making the vehicles safe from microbiological contamination (İETT 2015).

When we compared the results of the samples taken in morning and evening by public transportation vehicles, it was determined that the samples taken in the evening from all vehicles were much higher than the counts of the samples taken in the morning for aerobic bacteria and fungus counts.

In particular, the total aerobic bacteria counts of the samples taken in the morning from the buses were high so daily cleaning procedures were insufficient. In addition, the isolation of pathogens such as *S. aureus*, Staphylococcus and Enterococcus in the specimens shows that these vehicles may be important sources of infection.

The presence of *S. aureus* in these vehicles is of great importance for both hospital-acquired and community-acquired infections. Transmission is usually through direct contact or droplet contact. In humans, skin inflammation can lead to various organ infections such as wound infections, urinary tract infections, pneumonia or poisoning (Bilgehan 1992; Gürler 2008; Winston and Chambers 2009). Enterococci are among the leading endocarditis agents and may cause various infections such as bacteremia, salpingitis, endometritis, peritonitis, biliary tract infections, intraabdominal abscess, and sometimes meningitis (French 2010).

We have found that the microbial load in public transportation vehicles is high. It has also been shown that these vehicles can be an important source of contamination for many community-based infections. Our results suggest that these vehicles need to be cleaned, disinfected and tested more often. In addition, those who use these vehicles could prevent many diseases with proper hand washing techniques.

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Original Article

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The ethnobotanical notes from Nizip (Gaziantep-Turkey)

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ABSTRACT

This paper reports folk medicinal and food plants of Nizip (Gaziantep) located in the south part of Turkey. The purpose of the study is to gather, determine, and record the plants that are used as source of food and medicine by local people. The information was obtained from participants in face to face interviews; furthermore, the specimens of the plants were collected. Voucher specimens were deposited at the Herbarium of the Faculty of Pharmacy, Marmara University. Totally, twenty-seven plants are recorded as used as traditional folk medicine for the region, and thirteen of these are also used as a source of food. Among them, 20 taxa were wild and 7 taxa were cultivated plants. According to the majority of the plants which have similar usage, the plants are mostly used for gastrointestinal, respiratory system diseases and diabetes.

Keywords: Ethnobotany, folk medicinal plants, food plants, Nizip, Turkey

INTRODUCTION

Turkey has a rich traditional culture because the country is located in region where the effect of lots of civilization was observed. The important part of this culture include nutrition plants that can be eaten and folk medicine (Bulut, 2016). This riches is displayed with ethnobotany researches which is done in different regions of Turkey. Nizip where we performed preliminary researches take part in the east of Turkey but there isn't enough work in the region (Akan et al. 2008; Altundağ and Öztürk 2011; Bulut et al. 2016; Çakılcıoğlu and Türkoğlu 2010; Çakılcıoğlu et al. 2010, 2011; Doğan and Tuzlacı 2015; Kaval et al. 2014; Mükemre et al. 2015; Özgen et al. 2004; Özgökçe and Özçelik 2004; Sezik et al. 1997; Şığva and Seçmen 2009; Tabata et al. 1994; Tetik et al. 2013; Tuzlacı and Doğan 2010; Yeşil and Akalın 2009).

Nizip is situated (37°00'36"N 37°47'50"E) in the southern part of Turkey at an altitude 400 m above sea level (Figure 1). It covers an area of 1.031 km^2 and its population is 109.285. Nizip is surrounded by Yavuzeli in the north, Karkamış in the south, Birecik (Şanlıurfa) in the east and Şehitkamil and Oğuzeli in the west. The main crops of Nizip are olive and pistachio. Also, Zeugma ancient city that is located 10 km from Nizip has a historical importance in the region.

MATERIALS AND METHODS

This ethnobotanical study addresses the use of wild plants as a source of food and medicine. The study was made in 2012 and its materials were the plants (27 taxa) collected during the field work. The information was obtained through open and semi-structured interviews from the local people (Alexiades 1996; Cotton 1996; Martin 1995) with local people. The interviews were made as general conversations with a strict questionnaire (Appendix 1). The information about the local names, the part(s) used, the ailments treated, the therapeutic effect, the preparation, the methods of administration, and the duration of treatment was recorded. The "Flora of Turkey and the East Aegean Islands" (Davis 1965-1985; Davis et al. 1988; Güner et al. 2000) were mainly used for the identification of the plants. The plant specimens are kept in the Herbarium of the Faculty of Pharmacy, Marmara University (MARE).

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Table 1. Medicinal and food plants of Nizip (Gaziantep-Turkey)

Botanical name, Family and Specimen number	Local name	Plant part used (medicine)	Ailments treated/ Therapeutic effect	Preparation and Administration	Plant part used (food)	Modes of consumption	References
Alcea dissecta (Baker) Zohary (Malvaceae, MARE 15333)	Hatmi çiçeği	Flowers Flowers	Cough Gastro-intestinal disorders	Infusion, int. Infusion, int.			(15, 2)[b]
Astragalus lamarckii Boiss. [*Astracantha lamarckii* (Boiss.) Podl.] (Fabaceae, MARE 15337)	Geven	Aerial parts	Cold	Infusion, int.			
Capparis spinosa L. (Capparaceae, MARE 15329)	Kember	Gemmae Flowering branches	Rheumatism Expectorant	–, eaten Decoction, int.			(2)[b]
[a] *Cupressus sempervirens* L. (Cupressaceae, MARE15345)	Çam	Leaves & branches Cones	Constipation Enuresis nocturna	Decoction, int. Decoction, int.			
Ficus carica L. subsp. carica (Moraceae, MARE 15356)	İncir	Latex Dried fruits	Wart Constipation	–, ext. –, ext.	Fruits	Eaten raw, jam	(2)[b]
[a] *Hibiscus esculentus L.* [Abelmoschus esculentus (L.) Moench] (Malvaceae, MARE 15353)	Bami	Flowers Flowers	Constipation Gastro-intestinal	Decoction, int. Decoction, int.	Fruits	Cooked	
[a]*Juglans regia L.* (Juglandaceae, MARE15351)	Ceviz	Leaves Seed	Diabetes Dysmnesia	Decoction, int. –, eaten	Seed	Eaten raw	Diabetes (14) (2, 6, 8, 10, 11, 13, 15, 16)[b],
Lactuca serriola L. (Asteraceae, MARE 15338)	Şirok	Aerial parts Aerial parts Aerial parts Aerial parts	Liver diseases Kidney ailments Digestive Expectorant	Decoction, int. Decoction, int. Decoction, int. Decoction, int.			(4, 12, 14,)[b]
Mentha longifolia (L.) Hudson subsp. *typhoides* (Brig.) Harley var. *typhoides* (Lamiaceae, MARE 15335)	Punk	Aerial parts Aerial parts Aerial parts	Cold Appetizer Gastrointestinal disorders	Infusion, int. Infusion, int. Infusion, int.	Aerial parts	Spice	Cold (2, 10, 13) Gastrointestinal system diseases (16, 11) (3, 4, 5, 6, 8, 15)[b] Spice (3, 9)
[a]*Olea europaea* L. var. *europaea* (Oleaceae, MARE 15336)	Zeytin	Leaves Leaves Leaves Fruits	Appetizer Diabetes High cholesterol Constipation	Decoction, int. Decoction, int. Decoction, int. Olive oil, eaten	Fruits Fruits	Eaten raw Pressed into oil	(11, 12)[b]
Pistacia khinjuk Stocks (Anacardiaceae, MARE 15352)	Menengiç	Fruits	Cold	Ground and make a coffee, int.			(1)[b]
[a]*Pistacia vera* L. (Anacardiaceae, MARE 15346)	Antep fıstığı	Fruit Resina	Diabetes Stomach ulcer	–, eaten –, int.	Seed	Eaten raw	(12)b1 Eaten raw (12)
Platanus orientalis L. (Platanaceae, MARE 15355)	Çınar	Leaves Leaves Leaves	Calcification Antipyretic Toothache	Decoction, int. Decoction, int. Decoction, gargle			(12,14)[b]
Portulaca oleracea L. (Portulacaceae, MARE 15332)	Pirpirin	Aerial parts	Gastrointestinal disorders	Decoction, int.	Aerial parts Aerial parts	Boiled then salad (+ yogurth) Salad	Gastro-intestinal disorders (14) (2, 3, 5)[b] Salad (3, 12)
Punica granatum L. (Punicaceae, MARE 15348)	Nar ağacı	Seed Seed Seed	Constipation Cold Anthelmintic	–, eaten –, eaten –, eaten	Seed Seed	Eaten raw Squeezed ("nar ekşisi") then added in salad	

Table 1. Medicinal and food plants of Nizip (Gaziantep-Turkey)

Botanical name, Family and Specimen number	Local name	Plant part used (medicine)	Ailments treated/ Therapeutic effect	Preparation and Administration	Plant part used (food)	Modes of consumption	References
Quercus brantii Lindley (Fagaceae, MARE 15344)	Palamut ağacı Mature fruits Mature fruits	Leaves Diabetes Diarrhea	Itching Crushed, int. Crushed, int.	Decoction, ext.	Fruit	Roasted	Diabetes (15)
Rhus coriaria L. (Anacardiaceae, MARE 15342)	Sumak	Leaves & branches Fruits	Diabetes Appetizer	Decoction, int. Decoction, int.	Fruit	Crushed then added in salad	(2, 6, 13)[b]
Rubus sanctus Schreber (Rosaceae, MARE 15347)	Böğürtlen	Fruits Fruits	Cough Respiratory system diseases	Jam, eaten Jam, eaten	Fruit	Eaten	Respiratory system diseases (14) (2, 5, 6, 12)[b]
Salix acmophylla Boiss. (Salicaceae, MARE 15341)	Biy ağacı	Leaves	Rhematism	Decoction, int.			
Solanum nigrum L. subsp. *schultesii* (Opiz) Wessely (Solanum decipiens Opiz) (Solanaceae, MARE 15343)	Köpek domatesi	Aerial parts	Eye diseases	Burned, steam bath			
[a]*Sorghum bicolor* (L.) Moench (Poaceae, MARE 15354)	Süpürge bitkisi	Fruits	Diarrhea	Roasted, eaten			
Teucrium polium L. (Lamiaceae, MARE 15338)	Murad	Aerial parts Aerial parts Aerial parts	Stomach diseases Appetizer Diabetes	Infusion, int. Infusion, int. Infusion, int.			Stomach diseases(15, 2) Diabetes (2, 6, 8, 14, 16)
Thymbra spicata L. var. *spicata* (Lamiaceae, MARE 15331)	Zahter	Aerial parts Aerial parts Aerial parts	Stomach diseases Appetizer Cold	Infusion, int. Infusion, int. Infusion, int.			(2)[b]
Tribulus terrestris L. (Zygophyllaceae, MARE 15339)	Pıtrak	Aerial parts	Kidney stones	Decoction, int.			(2, 11, 12, 14)[b]
Verbascum sp. (Scrophulariaceae, MARE 15340)	Zarmasi	Flowers & leaves Flowers & leaves Flowers & leaves	Wound Sore throat Cough	Crushed, ext. Infusion, int. Infusion, int.			
Vitex pseudo-negundo (Hausskn. ex Bornm.) Hand.-Mazz [*Vitex agnus-castus* L. –Lamiacaeae] (Verbenaceae, MARE 15330)	Süpürge bitkisi	Flowering branches	Cold	Decoction, int.			
Zea mays L. subsp. *mays* (Poaceae, MARE 15349)	Darı	Stylus	Kidney stones	Decoction, int.	Fruit Fruit	Boiled/ Roasted Grinded for making flour ("mısır unu")	Urinary system diseases (3) (10)[b]

Int.: Internal use; Ext.: External use; [a]Cultivated plant; [b]Different usage – Directly usage; (1) Akan et al. 2008; (2) Altundağ and Öztürk 2011; (3) Bulut et al. 2016; (4) Çakılcıoğlu and Türkoğlu 2010; (5) Çakılcıoğlu et al. 2010; (6) Çakılcıoğlu et al. 2011; (7) Doğan and Tuzlacı 2015; (8) Mükemre et al. 2015; (9) Özgen et al. 2004; (10) Özgökçe and Özçelik 2004; (11) Sezik et al. 1997; (12) Şığva and Seçmen 2009; (13) Tabata et al. 1994; (14) Tetik et al. 2013; (15) Tuzlacı and Doğan 2010; (16) Yeşil and Akalın 2009.

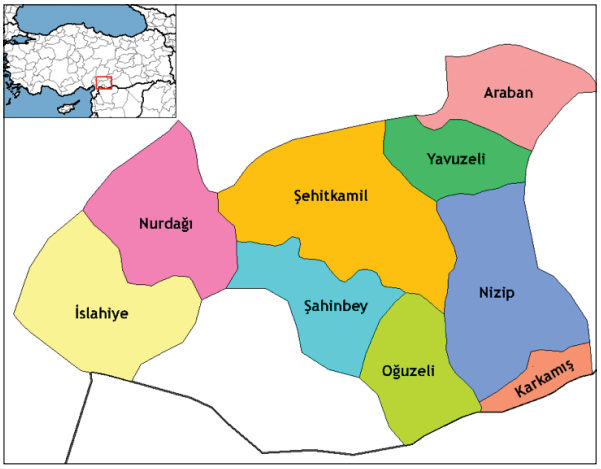

Figure 1. Map of Gaziantep (www.türkiyerehberi.com)

Figure 2. *Pistacia vera* L.

RESULTS AND DISCUSSION

The plants used for medicinal purposes in Nizip are presented in Table 1. Taxonomical changes according to the plant list (http://www.theplantlist.org) were shown in parenthesis in Table 1 together with the popular scientific names. Twenty-seven medicinal plant species, belonging to 11 families, were recorded in the research area. Of these, 20 taxa were wild, and 7 taxa were cultivated plants (Figure 2). The most common usages of the plants were found to be gastrointestinal, respiratory system diseases and diabetes.

The main preparation methods was decoction and usually aerial parts were used in the preparation.

We compared our results with other comprehensive ethnobotanical studies on folk medicinal plants which have already been carried out in the neighbouring areas (Akan et al. 2008; Altundağ and Öztürk 2011; Bulut et al. 2016; Çakılcıoğlu and Türkoğlu 2010; Çakılcıoğlu et al. 2010, 2011; Mükemre et al. 2015; Özgen et al. 2004; Özgökçe and Özçelik 2004; Sezik et al. 1997; Şığva and Seçmen 2009; Tabata et al. 1994; Tetik et al. 2013; Tuzlacı and Doğan 2010; Yeşil and Akalın 2009) and presented in Table 1. Among them, *Juglans regia* L., *Mentha longifolia* (L.) Hudson, *Rubus sanctus* Schreber and *Teucrium polium* L. recorded in eight localities were the most commonly used herbal medicinal plants in Nizip and its surroundings.

Thirteen taxa used medicinally (*Ficus carica* L. subsp. *carica, Hibiscus esculentus* L., *Juglans regia* L., *Mentha longifolia* (L.) Hudson subsp. *typhoides* (Brig.) Harley, *Olea europaea* L. var. *europaea, Pistacia vera* L., *Portulaca oleracea* L., *Punica granatum* L., *Quercus brantii* Lindley, *Rhus coriaria* L., *Rubus sanctus* Schreber, *Thymbra spicata* L. var. *spicata* and *Zea mays* L. subsp. *mays*) were also used as food plants.

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Appendix 1

Questionnaire Form

1. Name and surname of the participant
2. Age and sex of the participant
3. Telephone and address of the participant
4. Educational level of the participant
5. Date of interview
6. Place of residence of the participant
7. Duration of residence of the participant
8. Local name of the plant
9. Human health or animal health
10. Ailments treated /therapeutic effect
11. Plant part used
12. Preparation
13. Administration
14. Dosage
15. Duration of treatment
16 Age group of patients (baby, children, adults)
17. Side effect
18. Different ethnobotanical use

Original Article

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MLH1 -93G>A and I219V polymorphisms are susceptible to increased risk of sporadic colorectal cancer in a Turkish population

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ABSTRACT

Colorectal cancer (CRC) comprises approximately 10% of all cancers and is a major cause of cancer-related morbidity and mortality despite current diagnostic and treatment improvements. DNA damage and altered DNA replication through the deregulation of related genes cause genomic instability in sporadic CRC. DNA repair is very complex; many factors play a role to ensure that the restoration of errors occurs during the transfer of genetic material. MutL homolog 1 (*MLH1*) is one of the vital DNA repair genes responsible for genomic stability. Together with environmental factors, the genetic background may be associated with CRC development; thus, genetic polymorphisms are considered as risk factors. The present prospective case–control study aimed to determine the association between 93G>A and I219V polymorphisms of *MLH1* and CRC susceptibility in a Turkish population. The genotyping of 158 patients and 164 age- and sex-matched controls was performed by polymerase chain reaction-restriction fragment length polymorphism. Two variants, 93G>A and I219V, were associated with an increased risk of CRC. Individuals with A allele of 93G>A had an approximately 2-fold risk (OR: 1.92, 95% CI: 1.22–3.04; p<0.01) and those with G allele of I219V had an approximately 3-fold risk (OR: 2.82, 95% CI: 1.76–4.52; p<0.01) of developing CRC. Our results provide novel information for understanding the influence of *MLH1* on CRC risk in the Turkish population; however, further studies with a larger number of participants are required.

Keywords: MLH1 polymorphism, colorectal cancer, PCR-RFLP

INTRODUCTION

Colorectal cancer (CRC), for many years, is in the third place of all cancers both in men and women, and is a major cause of cancer-related morbidity and mortality (Berndt et al. 2007, Siegel et al. 2015). Genetic background covers a great position in the complexity of the CRC aetiology; and together with environmental factors, CRC incidence is varied among different populations (Aykan 2000, Haggar and Boushey 2009; Huxley et al. 2009). Recently, several pathways contributed to cancer development have been identified. It is well known that genomic instability is the origin triggers as well as other cancer-related mechanisms in hereditary cancers. However, in sporadic cancers, genomic instability is a consequence of DNA damage and alteration in DNA replication due to deregulation of the related genes in addition with impaired DNA repair (Anderson 2001, Negrini et al. 2010). Therefore, DNA repair mechanisms play crucial role in cancer prevention through maintaining genomic stability and DNA integrity (Abbas et al. 2013).

DNA repair is a very complex process in which many factors play a role together starting from the identification of the damaged region to post-replication. Mismatch repair (MMR) is one of the major mechanisms in DNA repair and MMR genes play

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a key role in fixing several errors such as insertions and deletions that occurred during the replication (Milanowska et al. 2010). *MutL Homolog 1* (*MLH1*), a member of MMR genes, has been suggested to be associated with both hereditary and sporadic CRCs through either genetic polymorphisms or epigenetic regulations (Richter et al. 2014, Zhang et al. 2016, Savio et al. 2017). For over a decade, the association between *MLH1* polymorphisms and CRC has been investigated; however, a definite conclusion has not been agreed (Burmester et al. 2004, Yu et al. 2006, Rajender et al. 2010, Valentin et al. 2012). It should be considered that conflicted results may be due to diversities between populations as well as numbers of the participants; therefore, further studies conducted with different populations were needed. In this present study, effects of *MLH1* polymorphisms on sporadic CRC susceptibility were evaluated in a Turkish population for the first time.

MATERIALS AND METHODS

A prospective case-control study was conducted with Turkish participants from Hospital of İstanbul University between 2011 and 2016. Adenocarcinoma of the colon or rectum was confirmed by routine laboratory and histological evaluations in 158 participants. Ethnic-age-sex-matched controls who had no history of any type of cancer were selected randomly from various divisions of the hospital and 164 participants were evaluated. All participants were provided informed consent. Study was conducted in accordance with the Helsinki Declaration and was approved by the ethics committee of İstanbul University (2016/1238).

Venous blood was collected into vacutainer EDTA tubes and within the same day genomic DNA was extracted by standard phenol-chloroform extraction protocol. DNA concentrations were measured by Take3 Plate on Epoch microplate spectrophotometer (BioTek, Winooski, USA) and diluted as appropriate. Genotyping of -93G>A and I219V was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Primers were synthesized by Sentromer DNA Technologies Laboratory (İstanbul, Turkey) and restriction enzymes were purchased by New England Biolabs (Hitchin, England). All other chemicals and plastic-ware were obtained from Sigma-Aldrich (St. Louis, Missouri, USA), Merck (Darmstadt, Germany) and Isolab GmbH (İstanbul, Turkey) as suitable grade for molecular research. Amplification and restriction conditions were shown in Table 1. Restriction products were identified on 2% agarose gels stained with ethidium bromide by a UV transilluminator. In each run, controls of known wild-type, heterozygous, and mutant genotype were included to check the accuracy that yielded 100% concordance.

All statistical analyses were carried out by using Statistical Package for Social Sciences (SPSS, version 21.0) and GraphPad Prism (version 5.0a) programs. Hardy–Weinberg equilibrium was tested by using chi-square (χ^2). The dominant model

Table 1. Amplification and restriction conditions of studied SNPs

SNPs	Primers	Annealing	Restriction	Fragment length (bp)
-93G>A (rs1800734)	F:5'-ACAgAgTTgAgAAATTTgACT-3' R:5'-ATCTCTTTgATAgCATTAgCT-3'	51.1°C	PvuII-HF 37°C 10 min	W/W: 180, 207 W/M: 180, 207, 387 M/M: 387
I219V (rs1799977)	F:5'-TCAgTACACAATgCAggCAT-3' R:5'-TACgTgAAATAAgAACTCCAT-3'	57.8°C	BccI 37°C 60 min	W/W: 345,107 W/M: 452, 345, 107 M/M: 452

SNPs: single nucleotide polymorphisms; rs: reference SNP number; F: forward; R: reverse; bp: base pair

Table 2. Genotype distributions of MLH1 polymorphisms among sporadic CRC cases and healthy controls

SNPs	Genotypes	Frequencies Cases (n=158, %)	Controls (n=164, %)	OR (95% CI)	p
-93G>A	GG	50 (31.6)	75 (48.1)	GG vs. any A	p<0.01
(rs1800734)	GA	60 (37.9)	66 (42.3)	1.92 (1.22-3.04)	
	AA	48 (30.5)	15 (9.6)		
MAF		0.494	0.307		
I219V	AA	42 (28.5)	87 (53.1)	AA vs. any G	p<0.01
(rs1799977)	AG	72 (48.9)	72 (43.9)	2.82 (1.76-4.52)	
	GG	33 (22.6)	5 (4.8)		
MAF		0.469	0.250		

SNPs: single nucleotide polymorphisms; rs: reference SNP number; MAF: minor allele frequency; OR: odds ratio; 95% CI: 95% confidence intervals

which is the wild type was chosen as reference group was used in genotyping analysis. Data comparisons were done by using Fisher's exact test; and, the odds ratios (ORs) and 95% confidence intervals (CIs) were estimated to evaluate the association between cases and controls. A two-tailed $p<0.05$ was considered to indicate a statistically significant difference.

RESULTS

MLH1 -93G>A and I219V polymorphisms were evaluated for the sporadic CRC susceptibility with the Turkish participants. All samples were genotyped with at least 93.1% successful rate and 100% concordance. There were no significant differences between cases and controls for age and sex, and genotype distribution was found to be consistent with the Hardy-Weinberg equilibrium ($p>0.05$) suggesting that the studied population was unbiased.

Genotype distributions of studied SNPs were summarized in Table 2. The -93G>A and I219V minor allele frequencies were 0.494 and 0.469 in cases whereas 0.307 and 0.250 in controls, respectively. The variant alleles of each SNP were compared between cases and controls based on the dominant model to estimate the risk of CRC. Individuals with *A* allele of -93G>A had about 2-fold (OR=1.92; 95% CI=1.22-3.04; $p<0.01$) and *G* allele of I219V had about 3-fold (OR=2.82; 95% CI=1.76-4.52; $p<0.01$) risk of developing sporadic CRC.

DISCUSSION

MMR system is responsible for impeccable DNA replication. As a result of inability to correct the errors during DNA replication, the length of microsatellite alleles changes due to the insertion and deletion of repetitive units in DNA. *MLH1*, a member of MMR genes, is one of the most studied genes due to its crucial role in maintaining the genomic stability through DNA repair. Genetic polymorphisms alone or together with epigenetic regulations in *MLH1* may cause microsatellite instability (MSI); thus may alter the cancer susceptibility (Berndt et al. 2007, Curtin 2012).

Because of the genomic instability is the initial factor in hereditary cancers, many researchers focused on the role of *MLH1* polymorphisms in Lynch Syndrome (LS) and/or Hereditary Nonpolyposis Colorectal Cancer (HNPCC) susceptibility. Rajender et al. (2010) reported that *MLH1* R659X (1975C>T) mutation is associated with HNPCC and 655A>G locus is highly polymorphic whereas I219V has no influence in Indian population. Valentin et al. (2012) found that 51.61% of the Southern Americans with LS carry Val allele of *MLH1* I219V; however, Val allele is not associated with CRC development. Colorectal and prostate adenocarcinomas coexist in the vast majority of HNPCC patients. Burmester et al. (2004) reported that *MLH1* I219V (A>G) variant is associated with prostate cancer; however, Fredriksson et al. (2006) found any association. Additionally, Fan et al. (2007) conducted a study with both hereditary and sporadic CRC patients in East Asia, and reported that *MLH1* V384D (T>A) and Q701K (C>A) polymorphisms might increase the risk. Also, Ohsawa et al. (2009) and Peng et al. (2015) reported that *MLH1* V384D polymorphism might be associated with sporadic CRC in Japanese and Chinese populations, respectively.

It is well known that, MSI is occurred in the half of the LS patients (Tantoğlu 2012). Campbell et al. (2009) suggested MSI-positive patients carrying -93A allele has 2-fold increased risk of colon cancer while there is no association between MSI-negative patients. However, in the same study, it was found that -93A allele is associated with smoking in MSI-negative patients. Additionally, Whiffin et al. (2011) reported that -93G>A polymorphism is associated with increased risk of both MSI-positive CRC and overall CRC.

Besides polymorphic changes, epigenetic regulations in *MLH1* may alter the risk of CRC. Mrkonjic et al. (2010) suggested that -93G>A polymorphism is associated with CRC in MSI-positive patients due to protein loss through methylation in the *MLH1* promotor region. Similarly, Kim et al. (2004) reported that I219V polymorphism and *MLH1* protein expression is correlated; however, Santibanez Koref et al. (2010) did not found any association between I219V polymorphism and *MLH1* methylation in CRC patients.

Our results suggested that both -93G>A and I219V variants are associated with 2-fold and 3-fold increased risk of sporadic CRC in Turkish population, respectively. Similarly, Allan et al. (2008) reported that -93A variant is increased the risk of CRC approximately 3-fold; and Wang et al. (2012) suggested that -93G>A polymorphisms increased the risk of MSI-positive CRC. In addition, Milanizadeh et al. (2013) suggested that *MLH1* I219V polymorphism is associated with sporadic CRC in East Asia.

On the other hand, Yu et al. (2006) reported that *MLH1* -93G>A polymorphism is not associated with colorectal adenoma and/or hyperplastic polyposis; however, -93A variant increased the 2-fold in the risk of adenoma and 10-fold in the risk of hyperplastic polyposis 10-fold among smokers. Similarly, Liu et al. (1995) and Peng et al. (2015) suggested that *MLH1* -93G> A, I219V and G39E polymorphisms were not associated with neither adenomatous polyps nor colorectal polyps. Also, Picelli et al. (2010) found that *MLH1* I219V polymorphism increased the risk of CRC risk; however, the association did not reach statistical significance.

CONCLUSION

The data is the first in understanding the influence of MLH1 on CRC risk in Turkish population, and suggested that -93G>A and I219V polymorphisms might be associated with sporadic CRC susceptibility. However, it should be considered that conflicted results may be due to diversities between populations as well as numbers of the participants; therefore, further studies conducted with different populations are needed.

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Short Paper

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Health beliefs and functional health literacy; Interaction with the pharmaceutical services

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ABSTRACT

Pharmacists are supposed to know current issues in social sciences and techniques to understand diseases and illnesses, to empathize with patients and other health professionals, to resolve possible conflicts of interest, to establish an ideal communication, to ensure the rational use of drugs, to reduce the wastage of drugs, and to improve compliance with drug therapy. The purpose of this article was to explain the conceptual framework of the Health Belief Model and Functional Health Literacy, which are recently outstanding topics on healthcare. Further, *Pharmacotherapy Literacy* is going to be defined as a remarkable subject in the literature. This is a descriptive study illustrating the concepts with the literature.

Keywords: Health beliefs, health literacy, pharmacotherapy literacy, public health

INTRODUCTION

Health Belief Model (HBM) has been developed by Hochbaum, Leventhal, Rosenstock and Kegeles in 1959, the United States of America, in response to the failure of a free or very low-paid Pap smear testing which was early detection of cervical cancer or Tuberculosis screening immunization program as important public health problem (Haefner and Kirscht 1970; Rosenstock 1974). Since then, HBM has been adapted to explain the relationship between beliefs and behavior of individuals about health and illness (Avcı, 2014).

Components of the HBM

There are six components of the model (Figure 1, Rosenstock et al. 1988; Çenesiz and Atak, 2007).

Perceived sensitivity: the perception of the disease that threaten the health of people; acceptance of the diagnosis, the probability of getting the disease.

Perceived severity: results will occur when the treatment not to be admitted; death, disease, disability, pain include assessment of the possible consequences, such as social losses. If the sensitivity and seriousness are taken together, this is defined as the Perceived threat.

Perceived benefits: due to perform behavior was perceived benefits associated with a reduced risk of developing the disease. People think that preventive health behaviors will give him/her the benefit. This benefit is expected to decrease the risk of developing the disease.

Perceived barriers: barriers to the realization of the proposed believed to difficult behavior or the potential negative aspects of the behavior. People weigh the positive and negative consequences of the behavior. The behavior is performed if perceived

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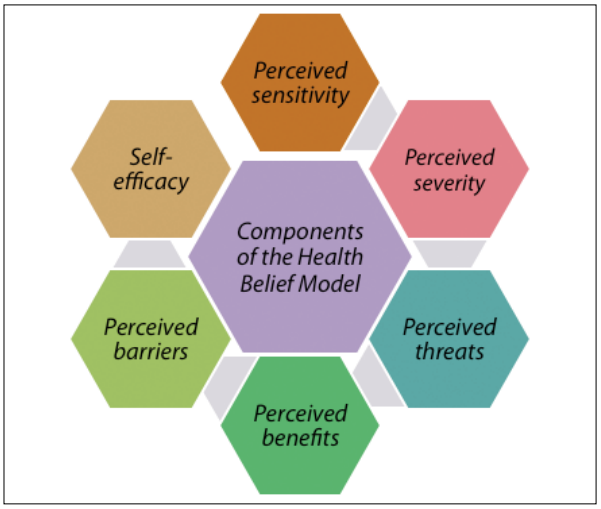

Figure 1. Components of the Health Belief Model (Rosenstock et al. 1988; Çenesiz and Atak, 2007)

susceptibility, severity and benefits reduce the impact of perceived barriers.

In other words, perception of health-related protective factors that prevent or make it difficult to perform a behavior. The most important variables that prevent the realization of protective health behaviors is the difference between the perceived benefits and perceived barriers.

Self- efficacy: one of the components of Albert Bandura's Social Cognitive Theory involves the person's self-belief, determination, self-control related the realization of behavior in order to achieve expected results. This component was added later to the model. Therefore, self-efficacy plays an important role in sustaining the introduction of changes in behavior and attitude.

For a better understanding of well-being, health concept is examined in two dimensions (Figure 2, Birol 2004). When considering the dimensions of health, the issue of health beliefs deserves special attention.

Belief areas that should be primarily discussed (Matthews and Hingson 1977)

A- Beliefs about the disease itself

This area include the beliefs of the seriousness of the disease and beliefs regarding the possibility of perceptual suffering from those in the future.

Beliefs can be released with some questions;

1- Why are you worried about high blood pressure?
2- How do you think about your pain now?
3- What do you think about the source of the problem you identify?
4- Do you know one who has cancer like you?

B- Beliefs regarding the benefits

The model evaluates patient's decision about the acceptance of a treatment plan in terms of cost-benefit analyzes. Many pa-

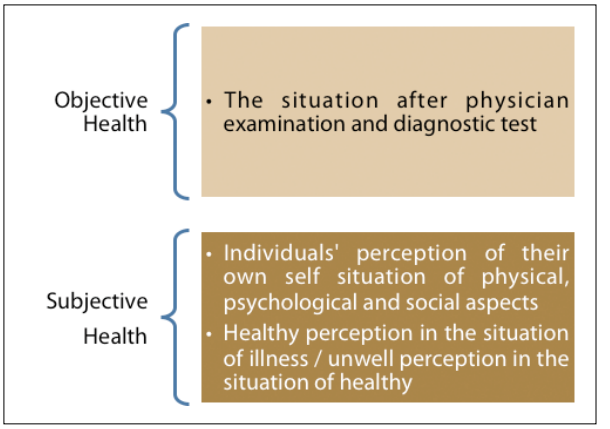

Figure 2. Health dimensions (Birol 2004)

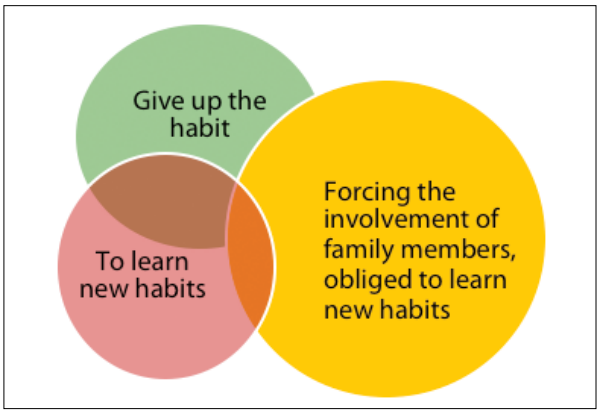

Figure 3. Reflection way of costs

tients who perceive significant cost associated with the treatment plan, are at risk of becoming poorly compatible while rejecting the slightest benefits of treatment. These costs sometimes means different things for the patient in finance (Figure 3).

The questions that would release the beliefs

1- What are you doing to yourself for your pain?
2- Do you think there might be any problems with these drugs when taken 1 hour before meals?
3- While monthly blood test, is there something bothering you?
4- Have you worried about side effects of this medicine?

The questions that would reveal benefits

1- Injections, how long do you think it would be dangerous for you?
2- If you forget to take their medication for a night, what do you think would happen?

Knowledge of health beliefs is important in terms of detecting the risk places of poor adherence patients and increasing patient compliance (Figure 4). Beliefs can be changed in important measure by each patient's visit. Health care workers are not the only source of health information for patients. Friends, relatives, magazines, mass communication-media, the diagno-

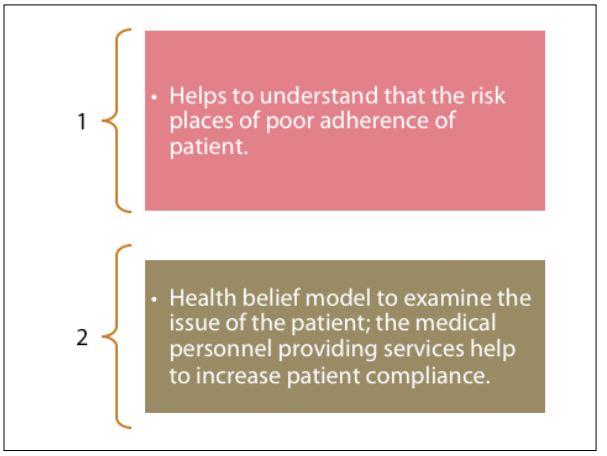

Figure 4. Why is it important to know beliefs? (Manheimer et al. 1973)

sis and treatment of disease may be a source of information in the process. All true or false information provided through these channels will play a role on the patient's compliance.

Health professionals would provide the patient's perspective and try to reveal patient's beliefs as well as to create the impression of patients. Afterwards impressed patients by more attention would respect health professionals with more confidence. These positive sensations could be perceived as a positive move by the patient and cause the patient's health behavior motivation.

Because of the well understood of the relationship between poor literacy skills and health status (Nutbeam 2008); health literacy, functional health literacy, and pharmacotherapy literacy (King et al. 2011) are the critical subjects for pharmaceutical services and pharmacists.

Literacy

The United Nations Educational, Scientific and Cultural Organization (UNESCO) established the Experimental World Literacy Program in 1966 and characterized literacy as being a fundamental human right. UNESCO defines literacy as "the *ability to identify, understand, interpret, create, communicate and compute, using printed and written materials associated with varying contexts. Literacy involves a continuum of learning in enabling individuals to achieve their goals, to develop knowledge and potential, and to participate fully in their community and wider society*" (Haefner and Kirscht 1970).

Health Literacy

Health literacy is an important issue in public health today, especially as patients are taking a greater role in obtaining information about their health (Manganello 2008). It is mentioned that inadequate health literacy is associated with poor health status, less medication adherence, lack of knowledge about disease, worse medical condition and earlier death (Baker et al. 1999; Eyüboğlu and Shultz 2015). Health literacy, according to the American Medical Asssociation (AMA) is that "*the constellation of skills involving performing such basic reading and numerical skills as reading medicine bottles and other materials related to health, and comprehending them- which is necessary for functionality in healthcare environments*" (AMA 1999).

Functional Health Literacy

Functional health literacy shows the basic skills of reading and writing about individuals' health. It emphasizes the ability of transfer the individual's health information into their lives (Nutbeam 2008; Aslantekin and Uysal 2014; Sağlık Bakanlığı 2014). "*Basic skills in reading, writing and "numeracy" are especially important in the healthcare setting, where patient participation in planning and implementing therapeutic regimens is critical for success"* (Parker et al. 1995).

Pharmacothearapy Literacy

The defenition of Pharmacotherapy Literacy was developed by King et al. because of the complex nature of the pharmacy-patient encounter. Pharmacotherapy Literacy is *"An individual's ability to abtain, evaluate, calculate, comprehend and properly act upon patient- spesific information concerning pharmacotherapy and pharmaceutical services necessary to make appopriate medication-related decesions, regardless of the mode of content delivery (e.g. written, oral, visual images and symbols"*(King et al. 2011).

CONCLUSION

Today, health care is located in a complex point where advanced technological developments in the health dimension are settled. The role of patients as consumers and individuals are intertwined. Also the individual's functionally health literacy in such a dynamic system is important. Individuals must be strengthened in this dynamic system by developing the level of their health literacy. Therefore, the health beliefs and functional health literacy which are important motivation in improving the health status of the community are critical scientific research topics. According to the scientific literature, health differences levels in community would be reduced by increasing the functional health literacy (Aslantekin and Yumrutaş 2014).

If one was identified as health illiterate, pharmacist would provide an education in terms of drugs. This education includes the use of both education aids and oral counseling methods to achieve the best outcomes of drug therapy (Tcakz et al. 2008). However, when we look at the scientific research conducted on the subject in the European Union between 1991-2005, Turkey ranks 14th with a total of 492 research (Sağlık Bakanlığı 2014). Eventually, first large-scale field research was applied by Sağlık Sen in 2014, Turkey (Sağlık Sen, 2014). According to the summary findings of this study;

- General health literacy index averaged over a 50-point scale was calculated as 30.4. The average index is 33.8 in Europe and the difference is significant. It is pointed out that 64.6% has insufficient or problematic health literacy. This figure corresponds to an adult population of 35 million. In other words, only 35.4% of the community has enough or excellent health literacy. This ratio is 52.5% in the European study.
- 51.7% of the population stated that they use drugs without medical advice or prescription.

- It has been shown that while the age is increasing and education level decreasing, health literacy linearly decreased.

Considering the results of the large scale research applied in Turkey, pharmacist can play a vital role in order to recognize the low health literacy or pharmaceutical literacy (Butler et al. 2013). According to a study focusing on glaucoma control has found that the level of medication adherence has been positively related to health literacy (Muir et al. 2006).

Pharmacists as one of the most accessable providers (Kehoe and Katz 1998) are supposed to be a part of the process of determining health illeterate patient in order to success the aimed results of drug therapy, especially when the half of the population use drugs without medical advice or prescription. Therefore pharmacy education curriculum or courses provided should include critical social subjects mentioned in the article.

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Review

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Antimicrobial peptides: Coming to the end of antibiotic era, the most promising agents

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ABSTRACT

Recently, because of the rising in multidrug resistance from infectious agents, there is a prompted interest for the development of new antimicrobial agents and new therapeutic strategies to combat the infections caused by the resistant bacteria. Among them, the natural bactericidal compounds, such as antimicrobial cationic peptides (AMPs) seems very promising agents. AMPs are the important component of the innate immune response to the surrounding microorganisms. These substances which can be isolated from most of the living organisms, have various activities, like broad spectrum antibacterial, antifungal, antiviral, and antiprotozoal. However there are some resistance mechanisms that affect the AMPs, because of the rapid action and existing more than one mechanism of action, development of resistance to AMPs is quite rare. Due to their many advantages and characteristics, AMPs looks like a good candidate for being a new generation, active antimicrobial agents for antimicrobial chemotherapy against especially multi drug resistant bacteria and biofilms, either alone or in combination.

Keywords: Antimicrobial peptides, antibiotic, resistance

INTRODUCTION

The misuse of antibiotics which are the main forces in antimicrobial therapy has led to the development of widespread resistance in microorganisms. Especially the nosocomial infections are caused by the Gram positive or Gram negative pathogens which have increased antibiotic resistance in intensive care units (Akova 2016; Rosenrhal et al. 2016). While some microorganisms are resistant to only one antimicrobial agent, many of them developed the resistance to multiple antimicrobials, so they called multidrug-resistant (MDR) strains. Infections caused by the MDR bacteria are generally not respond to antimicrobial therapy and they comprises a major risk for the mortality. Worse still, sometimes MDR microorganisms become resistant to all available antibiotics, named "pan-resistant organisms", and they could not treated with any single agent (Giamarellon 2010; Naim et al. 2016).

World Health Organization (WHO), had taken an extensive interest for that problem and published a report named "Antimicrobial resistance: Global report on surveillance 2014" to capture the attractions for antimicrobial resistance (WHO 2014). Then the "global action plan on antimicrobial resistance" was composed in 2015, by WHO as a World Health Assembly documents. That document outlines five objectives including improving the awareness and understandings, strengthening the knowledges and evidences, reducing the incidence of infections, optimizing the antimicrobial usage, and increasing the investment in new medicines (WHO 2015).

While combating the infectious diseases, there is conspicuous decrease in existing antibiotics, and as indicated by the WHO, finding of the alternative antimicrobial agents which have a new mechanism of action is very crucial. Antimicrobial activities of natural substances are always known, and there is a reinterest for their potential usages due to the rise of multidrug resistance in a variety of bacteria. Among them antimicrobial cationic peptides (AMPs) seems very promising antibacterial agents to controlling the resistant bacterial infections Donadio et al. 2010).

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This substances which, have AMPs are very prevalent in nature as important component of the innate immune response to the surrounding microorganisms, and they can be isolated from most of the living organisms such as insects, plants, microorganisms, mammals or non-mammalian vertebrates. AMPs have rapid action and various activity like broad spectrum antibacterial, antifungal, antiviral, and antiprotozoal (Hancock and Chapple 1999; Otvos 2005).

Structure of AMPs

AMPs are generally relatively short molecules, including 10–100 amino-acid residues display an positive net charge ranging from +2 to +11, and containing an amphiphilic and hydrophobic residues (in general 50%) (Hancock and Sahl 2006). Due to the increasing number of natural, semi-synthetic or synthetic AMPs, there are several databases are exist for today, which manage information and conduct peptide analysis (Wang 2015). As shown in Figure 1, despite their similar general physical properties, AMPs are classified based on the composition of their amino-acid, size and conformational secondary structures into major groups including peptides with β-strands, amphipathic α-helices, loop structure, and extended structures. Most of the AMPs are belongs to the first two categories (Hancock 2001; Jenssen et al. 2006).

Among AMPs, the most studied ones are colistin, melittin, indolicidin, nisin, CAMA, defensins, protegrins, magainins, etc. Colistin is a non-ribosomally synthesized AMP which is used as a prodrug as the methanesulfonic acid derivative of polymyxin E from ***Bacillus polymyxa*** var ***colistinus,*** and it's bactericidal to Gram negative bacteria especially ***Pseudomonas aeruginosa*** due to a detergent-like mechanism (Bechinger and Lohner 2006). Indolicidin is a haemolytic and antimicrobial peptide isolated from bovine neutrophils. It has a 13 tridecapeptide amide and an extremely high tryptophan content corresponding to its wide range of antimicrobial activities (Selsted et al. 1992). Nisin is an important bacteriocin AMP and one of the most studied lantibiotic which has a 34 amino acid including lanthionine and methyl-lanthionine. It is isolated from the bacteria ***Lactococcus lactis***, and shows rapid bactericidal effects against Gram-positive bacteria with no discrimination between multidrug resistant or sensitive pathogens due to its' dual mode of action against bacterial cell membranes (Willey and Van der Donk 2007). Melittin is a main toxic element of the honey bee venom, and an active AMP against Gram positive and Gram negative bacteria. It is known as a hemolytic peptide which have 26 amino acid and amphipathic α-helices (Raghuraman and Chattopadyay 2007). Because of the toxic characters of some effective AMPs, hybride peptides such as CAMA (Cecropin (1-7)-melittin A (2-9) amide) are designed for increasing the antimicrobial activity while decreasing toxicity. CAMA included the some amino acid regions of cecropin-A and melittin and is very active against Gram-positive bacteria, forming the ion-permeable channels in lipid membranes (Cao et al. 2010).

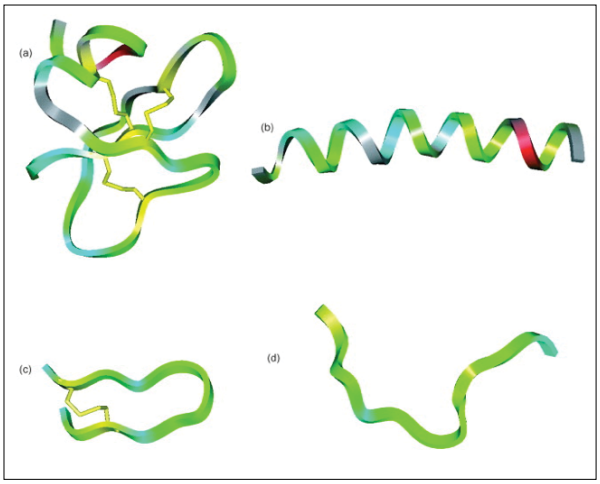

Figure 1. a-d. Structural classes of AMPs. (a) amphiphilic peptides with β-strands, (b) amphipathic α-helices, (c) loop structure, (d) extended structure

AMPs' mechanisms of actions

Most of the active AMPs interact with bacterial membranes, especially the Gram negative's lipopolysaccharide (LPS) layer. They create an ion-permeable channels, and increase membrane permeability to developing the cleavages. While crossing the small molecules into the bacterial cells from that cracks, some antimicrobial agents including the AMPs' are passing through the membrane. To explain how AMPs damages the membranes, as shown in Figure 2, a variety of possible membrane-weakening mechanisms such as toroidal, barrel-stave or carpet models have been maintained. As a result of this interactions, AMPs are taken up by "self-promoted uptake" pathway and they also affect many intracellular mechanisms to toward the bacterial cell death. The explanation both how AMPs bind to and inhibit endotoxins and how they shown the synergistic interactions with conventional antibiotics are existing by this pathway (Steinberg et al. 1997; Yeaman and Yount 2003; Wang et al. 2015).

Besides of the antimicrobial activities, AMPs also has an "enhancer" activity for classical antibiotics. That enhancement in activities of the antibiotics with appropriate AMPs, especially for MDR strains, caused by not only a permeability-increasing, but also the result of an increased access to the intracellular targets. Thus AMPs could be serve as anti-resistance compounds against planktonic cells (Sawyer et al. 1998; McCafferty et al. 1999).

On the other hand, when the planktonic forms of bacteria generally causes an acute infections, biofilm-associated forms cause persistent and chronic infections. A biofilm is a clusters of microorganisms in their extracellular matrix (EPS), on the biotic or abiotic surfaces. The cells growing in a biofilms are physiologically different from their planktonic forms. The bacteria in biofilms become more resistant to antimicrobial agents up to 1000-fold and the host's immune responses. That increased resistance in biofilm forming bacteria, could be explained by the decreased diffusion of antimicrobials, increased activity of multidrug efflux pumps, quorum-sensing systems, antimicrobial tolerance, and slow-growing cells, … etc (Donlan 2001; Høiby et al. 2010).

To prevent or delay the emergence of resistance caused by the biofilm, AMPs might be use in the antimicrobial combi-

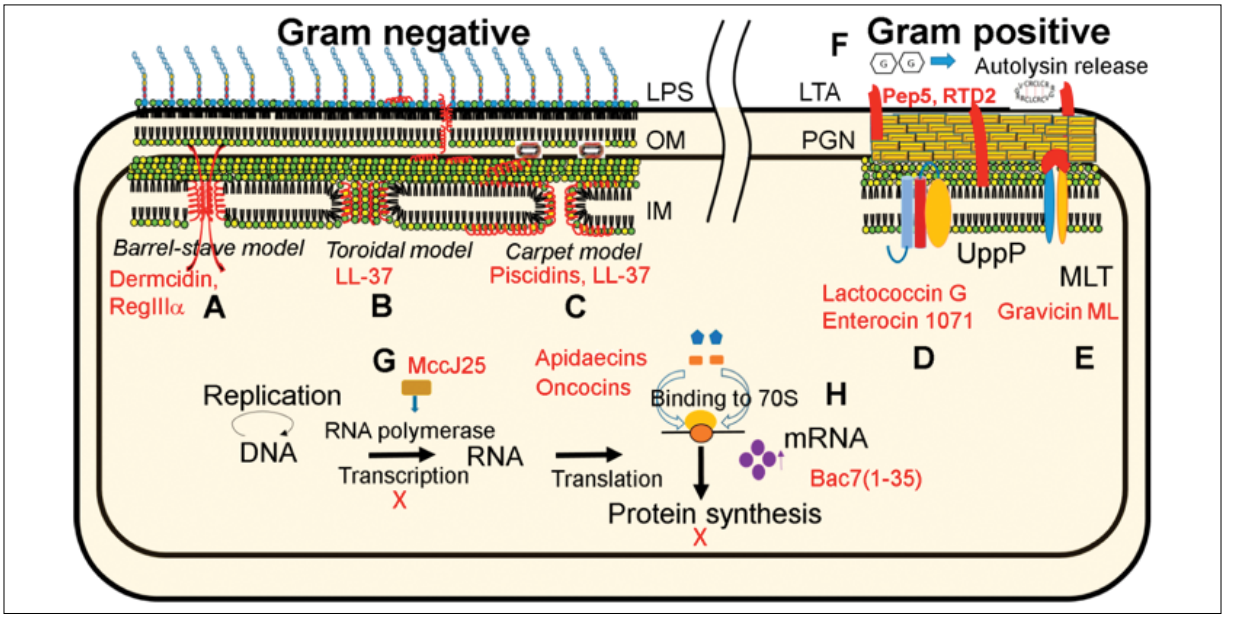

Figure 2. Mechanisms of action of AMPs (Wang et al. 2015).

nations to providing the synergistic interactions. There are many kind of AMPs were found active against bacterial biofilms in that way (Dosler and Mataracı 2013; Dosler and Karaaslan 2014). However, the anti-biofilm activities of AMPs are not completely understood, there are some possible explanations provided by some limited studies. That explanations includes matrix disruption; inhibition of some biofilm-related genes in DNA, blocking the quorum sensing systems, and AMPs dual mode of actioss on both the cytoplasmic membrane and intracellular targets (Hancock and Sahl 2006; Jorge et al. 2012).

AMPs' resistance mechanisms

AMPs are generally not affected by many resistance mechanisms that influenced the antibiotics. Also, there are a few number of resistance mechanisms which can effect antimicrobial cationic peptides and these mechanisms perform independently from others that effect antibiotics. A general mechanism causing resistance bacteria is the association of positively charged molecules onto the cell surface which reduces the interaction and binding of AMPs. The most important resistance mechanisms against AMPs are decreased permeability towards the cells, secretion of proteases, release of AMP degrading enzymes, down-regulation of host responses, active efflux, and alteration of the membrane physiology. In addition, evolutionary changes like mutations that allow innate expression of intrinsic resistance genes, also help for the bacteria to protecting from AMPs,. However there were some mechanisms that affect the AMPs, development of resistance to cationic peptides is quite rare because of the substantial theories such as "the death of the organisms occurs rapidly, hence, it does not leave much time for the bacteria to mutate and divide" and "AMPs possess multiple targets, hence, even if one fails, others remain to take the task forward" (Guilhelmelli et al. 2013; Nawrocki et al. 2014; Yeung et al. 2011).

AMPs' therapeutic potentials

Nowadays, there is a rising interest in potential therapeutic uses of AMPs. Especially linear and circular AMPs are preferable classes, because of the simple molecular structure and easy synthesis, and inherent stability to degrading by proteases, respectively. However the AMPs have desirable facilities for their therapeutic uses, there is some limitations especially lability depends on the environment including the presence of protease, pH change, and etc. for drug development. There are many ongoing studies to overcome the disadvantages that limits the potential clinical applications of AMPs. They generally focused on using the unusual amino acids such as D-forms, acetylation or amidation of the terminal regions, increasing the peptide stability against proteases, immobilization of AMPs on solid materials, using the modern drug delivery systems, such as liposome encapsulation or nanoparticles (Seo et al. 2012; Wang et al. 2015; Mendez-Samperio 2013). Some AMPs in clinical trials for therapeutic usage, are summarized in Table 1.

In conclusion, AMPs have attracted attention as alternative antibiotics due to their desirable properties such as potency, rapid and multiple mechanisms of action, broad spectrum activities, and low potential to induce novel resistance mechanisms, make them excellent prospects. There are a lot of clinical study which about local or systemic infections, showed that the AMPs can contribute to the rapid clearance of microorganisms through direct killing, inhibition of pro-inflammatory mediators such as lipopolysaccharide, and by modulating the inflammatory response to infection. However, in addition to ongoing phase II and phase III clinical trials, major concerns surrounding cationic peptides such as stability, toxicity, immunogenity, pharmacokinetic and pharmacodynamic parameters and antimicrobial activity will prove resolvability and they have to be formulated properly to develop into a drug form. According to those clinical trials,

Table 1. AMPs in commercial development

Peptide	Medical usage	Clinical trials
Pexiganan (MSI-78)	Diabetic foot ulcers	Phase III
Iseganan (IB-367)	Ulcerative oral mucositis	Phase III
Omiganan (MBI-226)	Bloodstream infections	Phase III
RDP58	Inflammatory bowel diseases	Post phase II
MBI-594 AN	Acne infections	Phase II
P113	Oral candidiasis	Phase II
P113D	Lung infections	Phase II
D2A21	Skin infections, burn wounds	Phase II
Neuprex (rBPI21)	Meningococcemia	Phase II
XMP 629	Topical dermal therapy	Phase II
hLF-1-11	Transplantation infections	Phase II
CZEN-002	Vulvo-vaginal candidiasis	Phase II
MX-226	Dermatological infections	Phase II
Glutoxim	Tuberculosis	Phase II
IMX-942	Immunomodulation, fevers in chemotherapy	Phase IA
HB-1345	Acne	Pre Phase I
Plectasin	Systemic anti Gram positive	Preclinical
HB-107	Wound healing	Preclinical

AMPs seems to be the new and active group of antimicrobial agent, not only as a single agent but also an adjuvant for the treatment of serious infections without a significant resistance problem in the near future (Hancock and Sahl 2006; Yeung et al. 2011; Findlay et al. 2016; Felício et al. 2017; Griffith et al. 2017).

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