Jundishapur Journal of Natural Pharmaceutical Products 2008; 3(1): 19-25

Jundishapur Journal of Natural Pharmaceutical Products

FORMULATION AND EVALUATION OF A NEW HERBAL TABLET FROM STRAWBERRY AND GRAPE LEAVES

Tavakoli N[1*], Ghodrati M[1], Ghassemi-Dehkordi N[2], Sadeghi-Aliabadi H[3]

[1]*Department of Pharmaceutics, School of Pharmacy and Pharmaceutical Science Research Center , Isfahan University of Medical Sciences, Isfahan, Iran.*

[2] *Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Science Research Center , Isfahan University of Medical Sciences, Isfahan, Iran.*

[3] *Department of Medicinal Chemistry, School of Pharmacy and Pharmaceutical Science Research Center , Isfahan University of Medical Sciences, Isfahan, Iran.*

Received: 9 March 2008 ***Accepted: 15 September 2008***

Abstract

Liver is the largest organ of the body, with several major functions. Increased consumption of chemicals (e.g. alcohol, bleach, preservatives, colorings, etc) and pharmaceuticals lead to a large number of liver diseases. Symptoms of liver malfunction include sudden exhaustion, bad taste in the mouth, loss of appetite, distaste foods, irritability or a general feeling of unhealthiness. There are a few herbal products from *Fragaria vesca* and *Vitis vinifera* as chewable tablets form in the market that are used as metabolic stimulator and as a treatment for all chronic inflammatory and degenerative liver conditions.

The leaves of grape and wild strawberry were collected, identified and dried. The content of anthocyanins present in the powdered leaves was measured based on a spectrophotometric differential pH method. To prepare chewable tablet (HepatoHeal), the same amount of powdered leaves of two plants (40 mg) was mixed to filler (mannitol or lactose) and granulated using wet granulation method. The resultant tablets were evaluated for hardness, friability, disintegration time, drug content uniformity, drug release test and organoleptic properties.

The assay showed that the content of anthocyanin in grape and strawberry leaves were 0.082(w/w) % and 0.039(w/w) %, respectively. The mean weight, friability, hardness, and disintegration time of selected formulation were 262 mg, 0.23%, 59.7 N and 22.6 min, respectively. The content of active ingredient (based on anthocyanin) was 44.8 mg and the content uniformity of the selected tablet was 42.8 mg. Percent of the drug released after 30 and 60 min was 76 % and 97 % respectively.

The selected formulation of HepatoHeal tablet has acceptable physicochemical features and may be considered as a herbal medication for some chronic inflammatory and degenerative liver disorders.

Keywords: Anthocyanin, Chewable tablet, *Fragaria vesca*, *Vitis vinifera*.

Introduction

Liver is the central organ for the detoxification and excretion of many xenobiotics including drugs. Partially all absorbable nutrients pass through the liver, which also implies that our nutritional well being largely depends on the liver (1, 2).

Chronic liver diseases represent a major health burden worldwide, with liver cirrhosis being the ninth leading cause of death in Western countries (3).

Even mild liver dysfunction can bring about excessive tiredness, bad taste in the mouth, lack of appetite and an aversion to certain foods (4, 5).

[*]E-mail: sadeghi@pharm.mui.ac.ir

Therapies developed along the principles of western medicine are often limited in efficacy, carry the risk of adverse effects, and are often too costly, especially for the developing countries. Therefore, treating liver diseases with plant-derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly practical. Furthermore, in spite of the advances in conventional medicine in the last decades, professionals and the lay public of developed countries pay increasing attention to phytomedicine(6).

Several recent surveys from Europe and the United States have demonstrated a sharp rise in the use of botanical drugs within a few years, and up to 65% of patients with liver disease take herbal preparations (7-9). Similar figures exist for Europe where the transactions for silymarin, a herbal preparation used to treat chronic liver diseases, reaches $180 million in Germany alone (10).

There is a public belief that herbal treatments are safe because they are natural and harmless comparing to conventional medicines. Supporters of herbal medicine claim that herbs may both treat and prevent disease (6). Moreover, herbal products are often free from rigorous regulations and prescriptions are usually not required for these inexpensive products.

Treating liver diseases with botanical drugs has a long tradition, but evidence for efficacy is sparse. In spite of some limitations about the quality of studies testing herbal remedies (e.g. unclear definition of exclusion and inclusion criteria, low statistical power due to small sample size and insufficient character-ization of used herbal preparations), a number of herbals (such as silymarin for antifibrotic treatment, phyllantus amarus in chronic hepatitis B and glycyrrhizin for treating chronic viral hepatitis) show promising effects, either experimentally in cell culture, in animal studies, or even in clinical trials (6).

There are a few herbal products from strawberry (*Fragaria vesca*) and grape (*Vitis vinifera*) as chewable tablets form in the market that are used as metabolic stimulator and a treatment for all chronic inflammatory and degenerative liver conditions, including chronic hepatitis, liver cirrhosis, constipation and eczema (11).

Grape has been used as an effective herbal remedy for liver disorders from many years ago and strawberry is known as a liver supporter herb. Getting these two healthful herbs together in a chewable tablet make it a suitable product for liver disorders because of their antioxidant and antiproliferative activities (12, 13).

In this study, a new formulation containing the same amount of strawberry and grape leaves, HepatoHeal chewable tablets, was produced and evaluated for its physicochemical and organoleptic properties.

Materials and methods

Materials

The grape and strawberry folium were collected from Koohpaye and Khansar cities, Isfahan province-Iran, respectively. Voucher specimens of the plants (No. 1779) were deposited at the herbarium of the school of pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran. Leaves were air dried in the shade, powdered and kept in tightened light protected container.

Mannitol, lactose, corn starch and magnesium stearate were obtained from Merck, Germany. Methanol and aspartame were purchased from Fluka chemical Co., Switzerland and NutraSweet, USA, respectively. Sodium acetate, potassium chloride, ethyl acetate, hydrochloric acid, sodium saccharine and all other chemical and reagents were USP-NF grade.

Hepatodoron®, a brand available in Europe by Weleda, was used as a control during in vitro tests.

Determination of Anthocyanins

Anthocyanins content of grape and strawberry folium were determined in dry powders by a pH differential spectrophotometric method (14). The same method was used for determining the active ingredient (anthocyanins) of tablets. A methanol- HCl (95:5) solvent system was used for extraction of anthocyanins. The pigment color depends on the pH of environment. Colored oxonium form of flavonoid exists at pH 1, however the colorless hemiketal form of flavonoid is predominant at pH 4.5. The difference in absorbance of these pigments at λmax of 510 nm is proportional to the flavonoid concentration. The withdrawn samples were diluted, using buffers of pH 1 and pH 4.5 and their absorption were determined in 510 and 700 nm, respectively. Anthocyanin concentration was calculated as cyanidin 3-glucoside equivalent by following equation:

$$\text{Anthocyanin pigments} = A \times M_w \times DF \times 1000 / \varepsilon \times L \quad \text{(as Cyanidin 3-glucoside, mg/L)}$$

Where A is the difference of anthocyanin absorbance, in two different medium, and calculated as $(A_{510\ nm} - A_{700\ nm})_{pH\ 1.0} - (A_{510\ nm} - A_{700\ nm})_{pH4.5}$,
M_w is the molecular weigh of Cyanidin 3-glucoside (= 449.2 g/mol),
DF is dilution factor,
L is the path length of cell (1cm),
ε is the molar absorptivity of Cyanidin 3-glucoside (= 26900 lit/ mol.cm),
1000 indicates the conversion factor of gram to milligram.

Tabletting and physicochemical evaluation of formulations

Four various formulations of HepatoHeal tablets were produced based on the same amount (40mg) of powdered leaves of two plants (Table 1). The active ingredients were mixed with fillers (mannitol or lactose), sweetening agent (saccharin or aspartame) and disintegrating agent (cornstarch) then granulated by wet granulation method, lubricated by magnesium stearate and finally were compressed by a rotary tablet press (Killian RU-35, Germany) to produce chewable tablets.
The resultant tablets were evaluated for pharmacopoeial characteristics such as hardness, friability, disintegration time, weight variation, drug assay, content uniformity and drug release test (15).

Taste assessment of tablets

The sensory panel test was performed with 10 volunteers, 20–24 years in age, for evaluation of chewable tablet taste. Randomly offered tablets were chewed in the mouth for 10 second and then mouth rinsed out by water. Tablets were rated from 0 to 5 points for their appearance, taste, sweetness, mouth feel sensation and aftertaste (16).

Table1: Composition of different formulations of HepatoHeal tablets

Tablet ingredients (mg)	**Formulation Code**			
	F1	**F2**	**F3**	**F4**
Grape leave powder	40	40	40	40
Strawberry leave powder	40	40	40	40
Mannitol	150	150	--	--
Lactose	--	--	150	150
Na Saccharine	0.5	--	0.5	--
Aspartame	--	2	--	2
Corn starch	5	5	5	5
Mg stearate	2	2	2	2
Starch paste	20	21	28	22

Overall tablets qualities were calculated as a sum of all recorded sensory properties. The obtained data were analyzed with the SPSS 10.0 statistical software. Significance of differences, at a 5% level, among means was determined by one-way ANOVA, using Post Hoc test.

Results and discussion

In this study HepatoHeal, a combination of *Fragaria vesca* and *Vitis vinifera*, has been formulated as a natural remedy for its hepatoprotective effects, liver regeneration and in combination therapy for hepatitis C (12, 13, 17).

Anthocyanins have been measured as active components of two plants. Their concentrations in grape, strawberry and mixture of two plants folium powder were determined 0.084%, 0.039% and 0.062% w/w respectively. Anthocyanins are unstable compounds in pure form, the availability of their standards is poor and their price is high (18). For determination of anthocyanins in glycoside form, it is very difficult to obtain standards for every anthocyanin present in the sample (19). The pH differential method has overcome these difficulties by using a known molar extinction coefficient. The basis of method is to measure the absorbance of two samples, diluted by buffers pH 1 and pH 4.5, in two different wavelengths (14, 20).

The result of hardness tablet test for selected formulation, including mannitol as filler and saccharin as sweetening agent, is 59.7N. Minimum acceptable level for hardness test is 40N for chewable tablets (16). The mean weight of selected tablet formulation was 262 mg and their weight variation is in the limits of U.S pharmacopoeia (Table 2). Friability of HepatoHeal tablets was measured to be 0.23% that is at the acceptable level of maximum 1% for tablets. Disintegrating time was also below 30 min (22.6 min) for the selected formulation (Table 2). Drug assay and content uniformity of the tablets (based on anthocyanin) was 44.8 and 42.8 mg respectively (Table 3). All measured tablets that comply with the test were between 85-115% of the average content. The amount of drug released after 30 and 60 min was 76% and 97% respectively. The profiles of drug release from HepatoHeal and Hepatodoron® tablets were compared in Fig. 1. Based on sensory panel test, the evaluation of various formulations of HepatoHeal tablets showed that there is no significant difference between appearance and hardness of different formulations. However, the tablet containing mannitol as filler (F1) was superior to that of F3 and F4 in term of mouth feel and sweetness. In addition, F1 and F2 had preferable aftertaste compared to F3 (Table 4).

Comparing organoleptic properties of Hepatodoron® and HepatoHeal tablets showed that HepatoHeal was more desirable than Hepatodoron® in sweetness and mouth feels.

Table 2: Weight variation, hardness, friability and disintegration time of different formulations of HepatoHeal tablets

Formulation Code	*Weight variation (mg)*	*Hardness (N)*	*Friability (%)*	*Disintegration time (min)*
F1	262.1 ± 6.8	59.7 ± 3.8	0.23	22.6 ± 1
F2	258.5 ± 8.8	61.3 ± 5.1	0.61	23.7 ± 2.4
F3	263.2 ± 7.3	76.2 ± 5.9	0.44	19.8 ± 0.8
F4	263.2 ± 6.2	64.7 ± 7.5	0.36	19.7 ± 1
Hepatodoron®	200.9 ± 2.5	28.8 ± 3.8	0.64	19.8 ± 1.2

Table 3: Drug assay (n=3) and content uniformity (n=10) of HepatoHeal and Hepatodoron® tablets

Tablet	*Drug assay (µg)*	*Content Uniformity (µg)*
HepatoHeal(F1)	44.8 ± 1.9	42.8 ± 2.7
Hepatodoron®	26.7 ± 0.8	25.7 ± 2.2

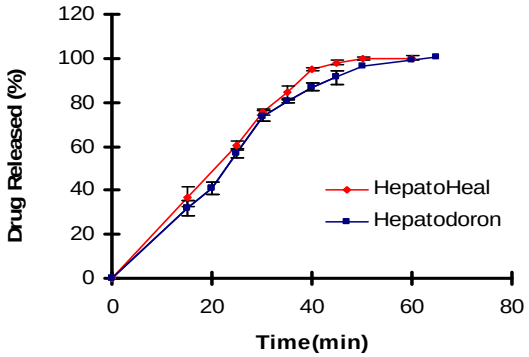

Fig. 1: Dissolution profiles of anthocyanins from hepatodoron and hepatoheal tablets.

Table 4: Sensory panel test for different formulations of HepatoHeal tablets

Attribute	Formulation codes				
	F1	F2	F3	F4	Hepatodoron®
Appearance	4.2 ± 0.63	4.0 ± 0.67	4.4 ± 0.52	4.4 ± 0.52	4.3 ± 0.64
Hardness	4.6 ± 0.51	4.5 ± 0.52	4.6 ± 0.51	4.2 ± 0.42	4.1 ± 0.7
Mouth feel	4.7 ± 0.48	4.1 ± 0.74	3.5 ± 0.85	3.6 ± 0.52	3.3 ± 0.9
Sweetness	4.4 ± 0.52	3.6 ± 0.70	3.8 ± 0.79	3.5 ± 0.71	2.8 ± 0.75
Aftertaste	4.5 ± 0.53	4.2 ± 0.42	3.2 ± 0.92	3.9 ± 0.57	4.0 ± 0.77
Overall	4.48	4.08	3.9	3.92	3.69

Score: 1(Poor), 2(Not good), 3(Acceptable), 4(Good), 5(Excellent)

Conclusion

Our data confirm that the selected formulation of HepatoHeal tablets has acceptable physicochemical features and may be considered as a herbal medication for some chronic inflammatory and degenerative liver disorders. These effects are mostly related to its major constituents, flavonoids, which has been demonstrated antioxidant and free radical scavenging properties.

Acknowledgment

Authors wish to thank the research council of Isfahan University of Medical Sciences for their financial support (No.384163). We also wish to thank Ms. Mahin Gharavi for her good suggestions.

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