Research paper

Design and evaluation of bioenhanced oral tablets of \textit{Dunaliella salina} microalgae for treatment of liver fibrosis

Farouk Kamel El-Baz\textsuperscript{a}, Sami Ibrahim Ali\textsuperscript{a}, Mona Basha\textsuperscript{b}, Ahmed Alaa Kassem\textsuperscript{b,\textdagger}, Rehob Nabil Shamma\textsuperscript{a}, Rania Elgohary\textsuperscript{d}, Abeer Salama\textsuperscript{a}

\textsuperscript{a} Plant Biochemistry Department, National Research Centre, El Buhouth St., Cairo, 12622, Egypt
\textsuperscript{b} Pharmaceutical Technology Department, National Research Centre, El-Buhouth St., Cairo, 12622, Egypt
\textsuperscript{c} Pharmacists and Industrial Pharmacy Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini St., Cairo, 11562, Egypt
\textsuperscript{d} Narcotics, Ergogenics and Poisons Department, National Research Centre, El Buhouth St., Cairo, 12622, Egypt
\textsuperscript{\textdagger} Pharmacology Department, National Research Centre, El Buhouth St., Cairo, 12622, Egypt

\textbf{ARTICLE INFO}

Keywords: \textit{Dunaliella salina}, Superdisintegrant, Dissolution, Bile salt, Thioacetamide, Liver fibrosis

\textbf{ABSTRACT}

The application of \textit{Dunaliella salina} (\textit{D. salina}) microalgae in nutraceutical products is limited due to poor aqueous solubility and consequently low bioavailability of the existing \(\beta\)-carotene. The aim of the present study is the design of bioenhanced oral tablets of \textit{D. salina} powder prepared by direct compression technique using the novel solubilizer, Sepitrap\textsuperscript{TM} 80, and bioenhancers as bile salts. The results showed that all prepared tablets manifested uniform diameter and thickness. The eligibility of the prepared tablets was confirmed by the friability test. The addition of the novel solubilizer, Sepitrap\textsuperscript{TM} 80, as well as crospovidone significantly reduced the disintegration time and improved the dissolution rate of \(\beta\)-carotene. Further enhancement was observed after the addition of bile salts. The oral antifibrotic effect of the optimized oral tablets of \textit{D. salina} powder was evaluated in a thioacetamide- (TAA-) induced fibrosis model in rats. The bioenhanced oral tablets of \textit{D. salina} powder reduced liver function enzymes, serum levels of interleukin-6, tumor necrosis factor-alpha, transforming growth factor-beta, hepatic contents of collagen-1, alpha-smooth muscle actin, elevated matrix metalloproteinase-9 significantly and finally improved the histopathological feature of hepatocytes. Hence, bioenhanced oral tablets of \textit{D. salina} powder have a promising antifibrotic potential against TAA-induced fibrosis in rats.

1. Introduction

Liver fibrosis is a progression of chronic liver diseases that affects the entire world population resulting in a high percent in morbidity and mortality after the development of liver cirrhosis [1]. It causes 1.3 million deaths, throughout the world, per year [2]. This disease induces the distortion of hepatic architecture, damages the normal function and enhances the deposition of excessive extracellular matrix (ECM) causing the development of cirrhosis or even hepatocellular carcinoma [3]. The activation of hepatic stellate cells (HSCs) plays a vital role in liver fibrosis progression [4]. Upon liver injury, the most potent fibrogenic cytokine, transforming growth factor-beta (TGF-\(\beta\)), provokes trans-differentiation of HSCs into active myofibroblast-like cells which express collagen-1 (Col-1) and alpha-smooth muscle actin (\(\alpha\)-SMA) [5]. At the same time, the activated HSCs secrete TGF-\(\beta\)1 that stimulates ECM production and up-regulates cell-matrix cell adhesion molecules [6]. On the other hand, some antifibrotic transcription factors become repressed during liver fibrosis. One of these important antifibrotic transcription factors is matrix metalloproteinase (MMP), that are secreted by kupffer cells (KCs) and has a central role in liver fibrosis resolution [7]. \textit{Dunaliella salina} (\textit{D. salina}) is one of the microalgae producing high amounts of carotenoids in nature. Carotenoids, the most prevalent pigments in nature, include mostly \(\alpha\)- and \(\beta\)-carotene, lutein, zeaxanthin, and lycopene. Principally, carotenoids in human healthy diets act as strong antioxidants and as vitamin A precursors [8]. \textit{D. salina} produce large amounts of \(\beta\)-carotene, well known for its several applications as a food colouring and a precursor of vitamin A [9]. The potent antioxidant activity of carotenoids encouraged researchers to investigate their potential. \(\beta\)-carotene exhibits the highest pro-vitamin A activity, and hence it is considered a powerful candidate for inclusion into dietary supplement products [10,11]. Therefore, \textit{D. salina} could be

\textsuperscript{\textdagger} Corresponding author. Pharmaceutical Technology Department, National Research Centre, El-Buhouth Street, Dokki, Cairo, 12622, Egypt. 
E-mail address: aa.kassem@hotmail.com (A.A. Kassem).

https://doi.org/10.1016/j.jddst.2020.101845
Received 31 March 2020; Received in revised form 19 May 2020; Accepted 2 June 2020
Available online 26 June 2020
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used to enhance free radical scavenging activities in the human body and save cells from oxidative harm [12].

The oral drug administration is the most convenient route and is the preferred one by patients considering the ease of administration, lack of pain and discomfort associated with the other routes. In order to develop bioactive molecules as therapeutic agents, increased oral bioavailability is a crucial consideration [13]. However, the oral administration of carotenoids generally and β-carotene specifically, faces a major problem represented in their very poor aqueous solubility thus hindering their dispersion, dissolution and subsequently absorption resulting in poor oral bioavailability [14]. Besides, the gastrointestinal tract (GIT) is considered a fundamental biochemical and physical hindrance to the systemic availability of orally administered therapeutic agents because of harsh enzymatic degradations and acidic environment in the stomach, mucus secretion, variable intestinal pH, etc. [15]. Previous studies have incorporated β-carotene in nanoemulsion systems to enhance its water solubility and bioavailability [16-18]. Compared to a bulk dispersion of β-carotene in the used oil, an increased retention of β-carotene in the emulsion, but with a lower bioavailability, was observed [14], in addition to chemical degradation after 28 days of storage [16]. Accordingly, there is an urgent need for the design of an appropriate formulation that can enhance the dispersibility, dissolution and absorption of β-carotene and thus optimize their bioavailability during GI passage.

Different approaches have been explored to improve the absorption and oral bioavailability of poorly soluble drugs, such as the use of novel excipients, chemical modifications, enzyme inhibitors, drug carriers, absorption enhancers, etc. [19]. The use of novel solubilizers and absorption enhancers represents the simplest strategy to improve the drug dissolution and permeation through the intestinal wall [20].

Sepitrap™ 80 is a new microencapsulated solubilizer exhibiting a very small particle size (<200 μm) and offered as free flowing powder. It comprises 45-65% of liquid polysorbate 80 adsorbed onto aluminosilicates carrier (35-55%). Sepitrap™ 80 enhances the polysorbate 80 characteristics via improving its bioenhancing and solubilizing potential because of the large surface area and by permitting incorporation of great quantity of polysorbate 80 into tablets (up to 10%) [21]. Polysorbate 80 is a commonly used non-ionic surfactant which enhances solubility and dissolution of poorly soluble drugs [22]. Bile salts are biomolecules that showed significant potential in drug delivery research owing to their favorable toxicity profiles and biological compatibility [23]. They hold a considerable advantage as drug absorption enhancers due to their ability to act as both permeation-modifying and drug solubilizing agents. Hence, bile salts can enhance bioavailability of therapeutic agents whose absorption-limiting factors include either low membrane permeability or poor aqueous solubility [24].

Thus, the objective of the present study is the design and evaluation of bioenhanced oral tablets of D. salina powder prepared by direct compression technique using the novel solubilizer, Sepitrap™ 80, and bioenhancers as bile salts. The effects of formulation variables on the different physicochemical characteristics in addition to the disintegration and dissolution behavior of tablets were investigated. Furthermore, biological evaluation of the antifibrotic effect was studied after oral administration of the developed tablets.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals and kits

Sepitrap™ 80 (microencapsulated solubilizer) was provided by Sepice S.A.France. Crospovidone (cross linked polyvinyl N-pyrrolidone, or PVP), cholic acid sodium salt 99% (SC), deoxycholic acid sodium salt 99% extra pure (SDC) and Aviceal (mircocrystalline cellulose) were purchased from Acros Organics, Belgium. Taurocholic acid sodium salt (STC) was bought from Loba Chemie, India. Thioacetamide (TAA) was procured from Sigma-Aldrich Co., USA. Aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin and albumin kits were obtained from Biodiagnostic Co., Egypt. Tumor necrosis factor–α (TNF-α), interleukin-6 (IL-6), transforming growth factor (TGF-β), collagen-1 (Col-1), alpha-smooth muscle actin (α-SMA) and metalloproteinase-9 (MMP-9) enzyme-linked immunosorbent assay (ELISA) kits were provided by SinoGenecom Biotech Co., Ltd., China. All other chemicals used in the study were of analytical grade.

2.1.2. Animals

Adult male albino Wister rats weighing 150–200 g were obtained from the animal house at the National Research Centre (NRC), Cairo, Egypt, and fed a standard laboratory diet and tap water ad libitum. Experimental animals were housed in an air-conditioned room at 22–25 °C with a 12-h light/dark cycle. All animals received humane care and the study protocols were carried out according to the ethical guidelines for care and use of experimental animals approved by the Medical Research Ethics Committee (MREC) at the NRC (Reg. No. 19/116).

2.2. Methods

2.2.1. Cultivation of D. salina in the vertical photobioreactor

D. salina was isolated from salt deposition basins of the Egyptian Salts and Minerals Company (EMISAL) and grown on Bold nutrient media [25] containing sodium chloride with a concentration of 100 g/l. The algal biomass was harvested and then grown in plastic bottles with a capacity of 17 l containing 15 l of microalgae culture with continuous aeration. After growing for 10 days, the culture was transferred to a fully automated and computer-controlled vertical photobioreactor with a capacity of 4000 l. Carbon dioxide was injected into the culture as a carbon source. The culture was left to grow until the biomass reached 2-2.5 g/l. Algal biomass was harvested by centrifugation at 2000 rpm for 15 min using basket centrifuge. Samples were washed twice with water, dried in an oven at 50 °C, ground into a homogenous powder and stored in a refrigerator for further chemical and biological investigation.

2.2.2. Preparation of algal extract

The dried biomass of D. salina (1 g) was soaked with 50 ml of ethanol/water (7:3, v/v) in 100 ml conical flask and kept on an orbital shaker (Stuart, England) at 160 rpm and room temperature for 48 h. The extract was filtered through filter paper Whatman No.1 to get the filtrate. The final volume of the filtrate was adjusted to 50 ml using measuring flask. One ml was filtered through a 0.2 μm syringe filter and then injected in the high performance liquid chromatography (HPLC) instrument for β-carotene determination. All the extraction steps were performed in dim light.

2.2.2.1. Analysis of algal β-carotene content by HPLC. D. salina extract was subjected to an analytical HPLC system equipped with an Agilent 5 prep-C18 Scalar column (5 μm; 150 mm × 4.6 mm) on an Agilent 1200 series instrument. The following solvents were used at a flow rate of 1.25 ml/min: (A) acetone and (B) methanol/water (9:1, v/v) containing 0.05% butylated hydroxytoluene (BHT). The separation of β-carotene was achieved by a gradient between solvents A and B for 40 min as follows: B was run at 80% for 25 min, 20% for 10 min, and 20–80% for 5 min. The separated β-carotene was identified using a photodiode array detector [26]. The peaks were integrated at 450 nm to quantify β-carotene. β-carotene (Sigma-Aldrich Co., USA) was used as standard. β-carotene in D. salina extract was identified and quantified through comparing retention time and the peak area of the unknown peak with the β-carotene standard.

2.2.3. Compression of tablets

Six different types of tablets were prepared by direct compression. Accurately weighed amount (555 mg) of the D. salina powder was mixed with the additives using mortar and pestle for 10 min, then the mixture
(750 mg) was manually filled into the die and compressed using single punch tablet press machine (Royal Artist, Mumbai, India) equipped with convex faced 13 mm punches, at a suitable compression force to obtain tablet hardness of about 4 ± 0.5 kg. The composition of the prepared tablets is listed in Table 1.

2.2.4. Characterization of the prepared tablets

2.2.4.1. Drug content. A tablet of each type was ground into a homogenous powder employing mortar and pestle and β-carotene content was measured using HPLC as previously discussed (section 2.2.2.), and calculated according to the following equation:

\[
\% \text{β-carotene content} = \frac{\text{Measured amount of β-carotene}}{\text{Theoretical amount of β-carotene added}} \times 100
\]

2.2.4.2. Uniformity of weight. The test was carried out according to the European pharmacopoeia [27]. Twenty tablets, from each formulation, were individually weighed and the mean of tablet weights was calculated.

2.2.4.3. Tablet friability. Twenty tablets, from each formulation, were accurately weighed and placed in the drum of a friabilator (Erweka type, GmbH, Germany). Tablets were rotated at 25 rpm for a period of 4 min and then removed, dedusted and accurately re-weighed. The percentage loss in weight was calculated and taken as a measure of friability.

2.2.4.4. In vitro disintegration time. Disintegration times of the prepared tablets were determined employing six tablets in each formulation, placed in the drum of a disintegration tester (Hanson S8, USA) at 37 ± 0.5 °C using a DST3 disintegration tester (Logan Instruments Corp., USA). The disintegration time was defined as the time necessary for the tablet to completely disintegrate until no solid residue remains or only a trace amount of soft residue remains on the screen. A digital stopwatch was used to measure the disintegration time to the nearest second. Only one tablet was analyzed at a time in order to ensure utmost accuracy.

2.2.4.5. In vitro dissolution study. The dissolution profile of β-carotene from the prepared tablets was determined in a dissolution tester (Hanson S8, USA) following the USP paddle method. All tests were conducted in ethanol/water (7:3, v/v) in order to ensure sink condition. Tablets were added to the dissolution medium, maintained at a temperature of 37 ± 0.5 °C with a paddle rotation speed of 100 rpm. At specified time intervals (0.5, 1, 2, 3, 4, 5, and 6 h), 5 ml of dissolution medium was withdrawn and replaced with an equal volume of fresh medium to maintain a constant total volume. Samples were filtered through 0.2 μm syringe filter and assayed for β-carotene content using HPLC at 450 nm (section 2.2.2.1.). Dissolution tests were performed in three vessels per formulation.

2.2.4.5.1. Calculation of dissolution parameters. The cumulative percent β-carotene released was plotted versus time and dissolution parameters i.e.; Q30 min (percent drug released after 30 min), initial dissolution rate, IDR (percentage dissolved of drug over the first 30 min per min) and relative dissolution, RDR (the ratio between amount of drug dissolved from optimized tablet formulations and that dissolved from the D. salina tablet formulation, F1, at 30 min) were calculated [28, 29]. Dissolution efficiency, DE (the area under the dissolution curve between two time points, t1 and t2 expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time) was calculated according to the following equation [30]:

\[
DE = \left( \frac{\int_{t_1}^{t_2} Y(t) \, dt}{Y(100) \times (t_2 - t_1)} \right) \times 100
\]

Where Y is the percent drug released as a function of time and Y100 is 100% drug release.

2.2.5. In vivo evaluation of the antifibrotic effect of D. salina tablets

After an acclimatization period of one week, male albino Wistar rats were randomly assigned to 5 groups of 8 rats per group. Group 1 were injected intraperitoneally (i.p.) with 1% Tween 80 (2.5 ml/kg) twice weekly for 6 weeks and then received distilled water (10 ml/kg; orally) daily for 4 weeks and served as normal control. Group 2 were injected i. p. with TAA (200 mg/kg; suspended in 1% Tween 80) twice per week for 6 weeks and then received distilled water (10 ml/kg; orally) daily for 4 weeks and served as TAA control according to Bai et al. [31]. Group 3 swallowed F1 tablets (100 mg/kg, orally) [9] daily for 4 weeks after TAA injection. Groups 4 and 5 swallowed F3 and F6 tablets (100 mg/kg, orally), respectively, daily for 4 weeks after TAA injection.

2.2.5.1. Collection of blood samples and liver homogenates. At the end of the experimental period, blood samples were withdrawn from the retro-orbital vein of each animal, under light anaesthesia. Blood was allowed to coagulate and then centrifuged at 3000 rpm for 15 min. The obtained serum was used to estimate the activities of AST and ALT, levels of total bilirubin and albumin. TNF-α, IL-6 and TGF-β were determined using ELISA.

Rats were then sacrificed by cervical dislocation and livers were excised, washed with saline and placed in ice-cold phosphate buffered saline (pH 7.4) to prepare 20% homogenate that was used for the estimation of Coll-1, α-SMA and MMP-9 using ELISA.

2.2.5.2. Histopathological examination. At the end of the experiment, part of the liver was removed carefully and fixed in 10% formalin for 24 h. Samples were washed under tap water, dehydrated in ascending

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Composition and characterization parameters of the developed D. salina tablets.</th>
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<tr>
<td><strong>Tablet code</strong></td>
<td><strong>D. salina powder</strong></td>
</tr>
<tr>
<td>F1</td>
<td>555</td>
</tr>
<tr>
<td>F2</td>
<td>75</td>
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<tr>
<td>F3</td>
<td>75</td>
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<td>F4</td>
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<td>F5</td>
<td>75</td>
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<tr>
<td>F6</td>
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Data are presented as the mean ± S.D. of (n = 6) for each parameter. Statistical analysis was carried out by ANOVA followed by Fisher’s LSD post-hoc test. For each parameter, same letter means non-significant difference, while different letter means significant difference at p < 0.05.
grades of ethanol (50, 70, 80, 90, and 100%) cleared in xylene, embedded in paraffin wax (melting point 55–60 °C). Liver sections of 4 μm thickness were prepared and stained with haematoxylin and eosin (H & E). Paraffin sections were stained in Harris's haematoxylin for 5 min. Sections were washed in running water then stained in 1% watery eosin for 2 min, washed in water, dehydrated, cleared and mounted in Canada balsam.

2.2.6. Statistical analysis

Results were presented as mean ± standard deviation (S.D.). Data were analyzed by one-way analysis of variance (ANOVA) followed by Fisher’s LSD post-hoc for means comparison using GraphPad Prism software, version 5 (GraphPad Inc., San Diego, USA). The difference was considered significant at p < 0.05.

3. Results and discussion

3.1. Analysis of algal β-carotene content by HPLC

Dunaliella microalgae have a high ability to synthesize high amounts of β-carotene in response to stressed growth conditions. In the present study, the HPLC analysis of ethanol/water (7:3, v/v) extract indicated that D. salina contains 159.33 μg/g β-carotene based on dry algal biomass. The commercial β-carotene (synthesized from different petro-chemicals) has only the all-trans form while the natural β-carotene is a combination of cis (mostly 9-cis and 15-cis) and trans-isomers. Living organisms absorb Dunaliella β-carotene, with its cis and trans-isomers mixture, better than the commercial one with its all-trans form [32].

3.2. Characterisation of tablets

The prepared tablets exhibited high percentage of β-carotene content (>97%). As presented in Table 1, all tablets manifested uniform diameter and thickness showing non-significant difference (p > 0.05) between them. All tablets revealed accepted weight variation range, with relative standard deviation of the tablet mass less than 1% for the developed formulations.

Consistent with compendial standards, tablets obey with the friability test if the weight loss during the test was <1% [33], in addition, tablets should not break or reveal any cracking or capping. The friability study confirmed the eligibility of the prepared tablets showing no cracks or chipped edges after tumbling. The calculated percentage weight loss was found to lie within the accepted range for tablets (<1%) signifying that the tablets were non-fragile and might be handled with ease.

Regarding the disintegration study, tablet formulations containing only D. salina powder (F1) and those comprising Sepitrap 80 (F2) exhibited long disintegration time (>27 min). On the other hand, the addition of crospovidone significantly (p < 0.05) reduced the disintegration time of tablet formulations (F3–F6) to less than 3 min. As a disintegrant, crospovidone swells in contact with water thus overcomes the adhesiveness of the tablet ingredients. In addition, this swelling increases the tablet porosity providing a pathway for liquid penetration by capillary action, and hence faster tablet disintegration [34].

3.3. In vitro dissolution study

Fig. 1 represents the in vitro drug dissolution profile of β-carotene from different D. salina tablet formulations. The obtained data clearly shows that after 6 h, only 41.81% of β-carotene was released from tablets prepared using Avicel only as a diluent and a binder (F1) with DE, Q50min and IDR of 34.16%, 26.52% and 0.88%/min, respectively (Table 2). This observed poor dissolution might be related to the very low aqueous solubility and the hydrophobic nature of β-carotene [35, 36] hindering the efficient wettability of the powder when added to the dissolution medium and consequently become dispersed on the surface preventing complete dissolution. Thus as an attempt to promote the solubility and consequently the dissolution of the powder, Sepitrap 80 was added (F2). The addition of the novel solubilizer, Sepitrap 80, resulted in a significant improvement in the dissolution rate of the drug, where 63.79% of β-carotene was released from tablets after 6 h and DE, Q50min and IDR increased to 51.10%, 35.82% and 1.19%/min, respectively (Table 2). The solid nature of Sepitrap 80 in the form of micro-encapsulated polysorbate 80 permits the incorporation of large amount of polysorbate 80 in the tablet matrix [21]. As a non-ionic surfactant, polysorbate 80 is commonly used to enhance the solubility and dissolution of poorly water-soluble drugs from solid oral formulations. At the same time, the presence of crospovidone in addition to Sepitrap 80 (F3) produced further enhancement in the dissolution rate of β-carotene (78.82% released after 6 h, DE, Q50min and IDR of 63.17%, 38.8% and 1.30%/min, respectively). This might be related to the porous structure of crospovidone as a superdisintegrant, which facilitated water uptake into the tablet resulting in increasing hydrostatic pressure and volume expansion causing tablet disintegration [37,38]. The obtained results are in accordance with in vitro disintegration data, where the presence of Sepitrap 80 and crospovidone enhanced the disintegration and accordingly producing faster drug dissolution.

Interestingly, the addition of bile salts resulted in a remarkable improvement in β-carotene dissolution profile where >65% was released within 30 min and more than 86.85% after 6 h. F4–F6 showed DE values of 76.27, 80.50 and 89.57% as well as Q50min of 65.26, 67.21 and 78.59%, respectively. Notably, their IDR and RDR were found to be 2.18, 2.24 and 2.62%/min as well as 2.47, 2.54 and 2.97, respectively (Table 2). The observed enhancement may be related to the specific surface active and interfacial properties of bile salts enhancing the
solubility of poorly water-soluble therapeutic agents mainly by the wetting effect [39]. Bile salts are characterized by a special chemical structure, having a rigid, large, and planar hydrophobic steroid nucleus with hydroxyl groups varying in number, orientation and position, with a flexible acidic side chain. They have been suggested to orient at the oil-water interface with the steroid backbone parallel to the interface, allowing the hydroxyl groups to interact with water molecules [19]. Accordingly, they can reduce the interfacial energy between the therapeutic agent and the dissolution medium, thus enhancing the effective surface area available for dissolution [40].

Previous attempts to increase the dissolution of β-carotene were reported. Salem et al. incorporated β-carotene in whey protein nanoparticles and found that nearly 30% of β-carotene was released after 2 h while a maximum release of 70% was observed after 8 h with a subsequent plateau maintained up to 12 h [41]. On the other hand, the release of β-carotene from zein-propylene glycol alginate composite nanoparticles didn’t exceed 34% after 3 h [42]. Teixe-Roig et al. reported 37% release of β-carotene from nanoemulsions after 2 h [43]. In another study, 60% β-carotene was released from canola oil-in-water emulsions after 2 h [44].

According to the obtained results, F6 composed of Sepitrap 80, crospovidone and STC attained the highest dissolution profile and dissolution parameters, thus was selected for biological evaluation along with F1 and F3 for comparison purposes.

3.4. In vivo evaluation of the antifibrotic effect of D. salina tablets

3.4.1. Assessment of hepatic function biomarkers

The present study has targeted the evaluation of the optimized D. salina bioenhanced oral tablet formulation (F6) compared to F1 and F3 tablets for management of liver fibrosis and exploring their mechanism of action to ameliorate this condition. TAA is a human carcinogen [45] inducing animal fibrosis similar to that occurring in human [46]. TAA generates oxidative stress [47] and activates HSCs leading to necrosis, apoptosis and excessive ECM proteins accumulation [48]. TAA damages liver cell membranes, causing cytoplasmic liver enzymes leakage into blood stream [49]. As shown in Table 3, TAA injection produced a significant elevation of serum ALT and AST activities as well as bilirubin levels (p < 0.05) with a decrease in serum albumin level. On the other hand, the administration of F1 tablets produced a significant (p < 0.05) decrease in serum activities of ALT, AST and bilirubin level as well as increased albumin level. In addition, F3 and F6 exhibited a significant decrease in levels of ALT, AST and bilirubin with an elevation in albumin serum level compared to TAA group. These findings are in good agreement with a previous work, which reported the protective effect of D. salina against liver injury and fibrosis via improving liver functions in Wistar rats [3].

3.4.2. Assessment of inflammatory biomarkers

Generally, TAA-induced fibrosis is associated with oxidative stress and increased pro-inflammatory cytokines expression: IL-1β, IL-6, and TNF-α [50,51]. TAA, also, elevated TGF-β [52] thus fueling fibrogenic and inflammatory reactions [53]. In TAA-treated rats, the obtained data depicted a significant rise (p < 0.05) in TNF-α, IL-6 and TGF-β serum levels by 62, 999 and 94% respectively, compared to the normal control group (Table 4). However, F1, F3 and F6 tablets significantly (p < 0.05) reduced serum levels of the hepatic pro-inflammatory cytokines; TNF-α by 28, 34 and 41%, IL-6 by 62, 76 and 89%, respectively, and TGF-β by 26, 31 and 35%, respectively, when compared to TAA group. These results propose the anti-inflammatory effect of D. salina as one of the possible mechanisms participating in its antifibrotic effect. In addition, the administration of F6 decreased liver contents of TNF-α, IL-6 and TGF-β by 12, 59 and 9%, respectively, compared to F3 group. In a previous report, D. salina down regulated inflammatory mediators and oxidative stress molecules with up regulation of antioxidant enzyme as thioredoxin and reduced glutathion in a streptozocin-induced diabetic neuropathy rat model [54].

3.4.3. Determination of hepatic contents of Col-1, SMA-α and MMP-9

Upon liver fibrosis, HSC is activated [55], becomes fibrogenic cell and coordinates ECM formation [56]. TGF-β1 is a potent HSC activator [57] secreting matrix proteins; Col-1 and α-SMA, that are considered as liver fibrosis key factors [58,59]. In addition, upon chronic liver injury, HSCs were activated and lost their capacity to store vitamin A [60] initiating collagen formation [61]. Fig. 2 illustrates excessive accumulation of matrix proteins resulting in liver fibrosis evidenced by the high liver contents of Col-1 and α-SMA, which increased significantly by 116 and 178%, respectively, in TAA group compared to the normal control group [62]. On the other hand, administration of F1, F3 and F6 tablets showed a significant (p < 0.05) decrease in liver contents of Col-1 by 12, 25 and 51%, and α-SMA by 43, 56 and 63%, respectively, compared to the TAA group. Moreover, the administration of F6 decreased liver contents of Col-1 and α-SMA by 35 and 16% compared to F3 group. These results indicated that D. salina β-carotene content ameliorates the loss of vitamin A which in turn inhibits collagen formation and fibrogenesis.

Other cells involved in fibrosis are KCs which are hepatic macrophages and expressed MMPs enzyme. These enzymes degrade collagen, induce ECM softening and reverse activated HSCs to quiescent state [63]. In the present work, TAA caused a significant decrease (p < 0.05) in MMP-9 by 94% compared to normal control group, while D. salina oral tablets; F1, F3 and F6 administration contribute to fibrosis resolution through elevating MMP-9 hepatic content by 5, 7 and 8 folds, respectively, compared to TAA group. The administration of F6 elevated MMP-9 liver content by 19%, compared to F3 group. In a previous work,
D. salina administration in higher dose than used in this study (200 mg/kg) upregulated MMP-9 hepatic content in fibrotic rats [64].

By over viewing the above results, it can be clearly depicted that all prepared D. salina tablets; F1, F3 and F6 revealed a noticeable improvement in TAA-induced liver fibrosis as evidenced by the enhanced effect on the measured markers at the end of the study. However, F3 and F6 showed superior effect compared to F1. The obtained results confirmed that the presence of crospovidone and Sepitrap 80 in both formulations promoted the efficacy of β-carotene through the recorded acceleration of the disintegration time, the improved dissolution profile and hence the penetration of β-carotene, leading to rapid and enhanced absorption and thus improved bioavailability. Moreover, the data noticeably show that F6 had a remarkable effect compared to F3, which might be attributed to presence of the bile salt, STC, and its impact on both drug dissolution and absorption. Generally, the solubility of lipophilic compounds is considerably increased in the presence of bile salts, resulting in a subsequent improvement in the absorption rate and bioavailability [19]. In the current study, the dissolution of β-carotene was significantly enhanced in the presence of STC as indicated by the dissolution study. Beside the former effect on the dissolution, owing to the amphiphilic nature, STC as bile salt can partition into the biological membranes, interacting with the phospholipid bilayer, changing the distribution of proteins and lipids and increasing the membrane fluidity and permeability, leading to enhancement of absorption and oral bioavailability [65]. The dual effect of STC might explain the observed promoted in vivo efficacy of F6.

3.4.4. Histopathological examination

As observed in Fig. 3, the liver section of the normal control group showed normal parenchymal hepatocytes and central vein (Fig. 3A). However, the liver sections of TAA group revealed fibroblastic cell proliferaions with inflammatory cells infiltration that are dividing the degenerated hepatocytes into lobules (Fig. 3B) associated with severe dilatation of the portal vein with periductal fibrosis surrounding the bile ducts (Fig. 3C). Regarding the treated groups, samples of F1 group showed centrilobular necrosis that was detected in the hepatocytes surrounding the central vein with inflammatory cells infiltration (Fig. 3D), while those of F3 group showed dilatation in the central vein with inflammatory cells infiltration (Fig. 3E). Interestingly, liver sample from F6 group showed few inflammatory cells infiltration with dilated central vein (Fig. 3F). The above histological findings signify the role of the D. salina oral tablets generally and F6 specifically in the management of liver fibrosis, controlling the expected liver damage.

4. Conclusions

From the results of the current work, it could be concluded that the fabricated tablets of D. salina powder proved uniform diameter and thickness as well as consistent durability against breakage. The addition of the novel solubilizer, Sepitrap™ 80 as well as crospovidone significantly reduced the disintegration time and improved the dissolution rate of the drug with further enhancement after the addition of bile salts. Bioenhanced oral tablets of D. salina powder revealed a significant antifibrotic effect in TAA-induced fibrosis in rats via ameliorating the elevation of liver enzymes, inflammatory mediators and fibrotic markers.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Farouk Kamel El-Baz: Conceptualization, Writing - review & editing, Project administration, Funding acquisition. Sami Ibrahim Ali: Investigation, Writing - original draft. Mona Basha: Conceptualization, Methodology, Formal analysis, Writing - review & editing. Ahmed Alaa Kassem: Conceptualization, Methodology, Formal analysis, Writing - review & editing. Rehab Nabil Shamma: Conceptualization, Methodology, Investigation. Rania Elgohary: Methodology, Investigation, Writing - original draft. Abeer Salama: Conceptualization, Methodology, Formal analysis, Writing - review & editing.
Acknowledgments

This work was supported by the alliance entitled “Integrated Pharmaceutical Alliance (IPA)” funded by the Academy of Scientific Research and Technology, Egypt under the “Egypt Knowledge and Technology Alliances (EG-KTAs) Program C2-2.10”.

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Fig. 3. Histopathological micrographs of rats’ livers stained with (H & E) (A): Liver section of rat from normal control group with normal histological structure of the central vein. (B) & (C): Liver sections of rat from TAA group with fibroblastic cells proliferations and inflammatory cells infiltration that dividing the degenerated hepatocytes into lobules associated with sever dilatation of the portal vein as well as periductal fibrosis surrounding the bile ducts. (D): Liver section of rat from F1 group with centrilobular necroses that detected in the hepatocytes surrounding the central vein and inflammatory cells infiltration. (E): Liver section of rat from F3 group with dilatation in the central vein and inflammatory cells infiltration. (F): Liver section of rat from F6 group with few inflammatory cells infiltration and dilated central vein.


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