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Preparation, characterization and anti-colitis activity of curcumin-asafoetida complex encapsulated in turmeric nanofiber

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Abstract

Ulcerative colitis (UC) is a main form of inflammatory bowel disease (IBD). Asafoetida (ASF) and turmeric have traditionally been used for the treatment of various inflammatory diseases, including UC, because ASF is rich in sulfur compounds and turmeric contains curcumin (CUR). Turmeric nanofiber (TNF), the modified cell wall component of turmeric is considered to play important role in the human diet, health and can be used as a carrier agent to encapsulate bioactive components. A novel gut health product (GHP) was formulated by encapsulation of ASF and CUR complex onto TNF. The GHP was characterized by UPLC, GC-MS, FTIR, XRD, SEM with EDS and DSC studies. GHP was evaluated for anti-colitis activity in a rat model of 5% dextran sulfate sodium (DSS) induced UC. Treatment with GHP significantly attenuated the disease activity index, colitis score, histopathological changes and myeloperoxidase activity. GHP has significant protective effects against DSS induced colitis.

**Keywords:** Gut health product; ulcerative colitis; turmeric nanofiber; curcumin; asafoetida; dextran sulfate sodium
1. Introduction

Gut health is a complex concept and it is proposed as three most important components, namely the diet, the mucosa, and the commensal flora. The mucosa is composed of the digestive epithelium, the gut-associated lymphoid tissue and the mucus overlying the epithelium. The gut-associated lymphoid tissue, commensal bacteria, mucus and host epithelial cells interact with each other, forming a delicate and dynamic equilibrium within the alimentary tract that ensures efficient functioning of the digestive system [1]. Ulcerative colitis (UC), which is the main form of inflammatory bowel disease (IBD), is a nonspecific inflammatory disease of the large intestine. In addition, it is a lifetime illness with intense emotional and social impacts, it causes severe intestinal tract damage and its development to chronic UC can lead to colon cancer [2,3].

Food and feed should be selected to favor conditions in the gut that create and stabilize this equilibrium between the host, the microflora, and environment, and to prevent trouble of the structure and function of the gut [1]. Dietary fiber (DF) has a marked effect on gut anatomy, gut development and gut function. DF is the main substrate for bacterial fermentation, particularly in the large intestine of human and also non-ruminant animals. As DF interacts both with the mucosa and the microflora, it has an important role in the control of gut health. In general, DF ingestion leads to the increased size and length of the digestive organs, including the small intestine, caecum and colon [4,5]. These effects are often associated with modification of the gut epithelium morphology, and consequently with the hydrolytic and absorptive functions of the epithelium. Mucins are the major glycoproteins of the mucus layer that coats and protects the gut from infection, and from physical, chemical and enzymatic injuries, and aids the passage of lumen contents through the tract. DF increases the excretion of mucin at the terminal ileum in
many species, including the pig, rat and man. DF modulates gut health by way of complex interactions with the gut epithelium, the mucus and the micro flora [1].

DF is a broad category of non-digestible cell wall component of plant materials that includes non-starch polysaccharides, oligosaccharides, lignin, and analogous polysaccharides with an associated health benefit [6]. The physical properties of DF vary, and even a slight variance may influence the physiological effect of the DF. DF is classified by solubility in water, viscosity and microbial fermentation in the large intestine. Soluble DFs include pectin, gums, and polysaccharides, whereas insoluble DFs include cellulose, hemicellulose and lignin [7]. Many studies suggest that there is an association between high DF intake and a low incidence of colon cancer by reducing the digestion, absorption of macronutrients and decreasing the contact time of carcinogens within the intestinal lumen [6,8,9] and that DF has anticancer properties [6,10,11]. Furthermore, the US Food and Drug Administration have approved health claims supporting the role of DF in cancer prevention [12]. DF increases fecal bulking and viscosity, reduces the time for proteolytic fermentation that results in harmful substances, and shortens the contact between potential carcinogens and mucosal cells. In addition, DF can bind/excrete potential luminal carcinogens, lower fecal pH in the colon, and thus provides a healthy intestinal environment. DF decreases the risk for type 2 diabetes mellitus, obesity, cardiovascular disease, colon cancer, and improves immunity by modulating the gut microbiota landscape [6]. DF modulates our health at nearly every level, and in every organ system, through complicated modes of action, many of which remain to be determined. DF bind potential nutrients, result in new metabolites, and modulate nutrient absorption/metabolism. DF consumption can have
significant health benefits, particularly in laxation, mineral absorption, potential anticancer properties, lipid metabolism and anti-inflammatory effects [11].

Turmeric is well known for its medicinal properties and most of the medicinal properties of turmeric have been reported to be due to the active principle curcuminoids viz. curcumin, demethoxycurcumin and bisdemethoxycurcumin which were found to be beneficial in improving many biological activities [13]. However, not much is known about the various applications of turmeric when curcuminoids are removed from the turmeric. The extracted curcumin from turmeric markets as a value added nutraceutical and the material that remains is a by-product called turmeric spent. This turmeric spent is rich in dietary fiber (45%) contains both soluble (2%) and insoluble (43%) fibers. Nanofibers (NF) and dietary fibers (DF) are well established to play a beneficial role against various diseases like diabetes, gastrointestinal disorders, colon cancer, heart disease, etc [14,15]. A number of research reports are available showing the development of nanofibers and blending through wide range of biodegradable natural or synthetic polymers loaded with bioactive molecules for their potential applications [16, 17], such as tissue engineering [18-21], wound dressing [22], drug delivery [23] and medical devices [16, 24, 25]. NF prepared from biomaterial, turmeric spent containing DF is called as turmeric nanofiber (TNF) can be utilized to improve gut health as well as used as an encapsulating agent of natural bioactive compounds for the treatment of a wide range of pharmacological activities particularly gastrointestinal health and anti-colon cancer.

Natural health products as functional foods are currently being investigated on a priority basis for potential health promotion and reduction of disease risks. Current research trends have focused on the encapsulation of bioactive compounds or bioactive natural extracts into
nanofibers as composites. Encapsulation is widely used in the food, chemical and pharmaceutical industry to develop new functional foods or formulations containing or incorporating bioactive phytochemicals and plant extracts to enhance food safety and promote health. It is used to protect active molecules against light, humidity and oxygen in order to avoid or delay their degradation and stabilize them during storage before use. It also allows limiting or controlling their transfer to the environment in order to avoid losses, to mask some of their properties such as taste, odor, catalytic activity or to get a controlled release at given time and place; and total or progressive dosed delivery [26].

Asafoetida (ASF) – [Ferula asafoetida] is an oleo-gum-resin and used as a flavoring agent in food and as a traditional medicine for many diseases in many parts of the world. It has a strong, tenacious and sulfurous odor and it is a popular ingredient in the Indian cuisine, most probably because its odor is reminiscent of the flavor of garlic and onion, two sprouting vegetables, as well as meat. ASF is traditionally used for the treatment of different diseases, such as whooping cough, asthma, ulcer, epilepsy, stomachache, flatulence, bronchitis, intestinal parasites, antispasmodic, weak digestion and influenza [27]. ASF is an effective remedy for several diseases of the stomach. The digestive stimulant actions of ASF are the most commonly experimented beneficial physiological effect via enhanced secretion of saliva and activity of salivary amylase. It plays an important role in the digestion of dietary lipids by stimulating bile flow and enhances the bile acid secretion and also enhances the activities of digestive enzymes of the pancreas and small intestine. Moreover, it is used for low acid levels in the stomach, stomach pressure, flatulence and loose stools. Recent pharmacological and biological studies have also been reviewed and shown several activities, such as antioxidant, antimicrobial,
antiviral, antifungal, cancer chemopreventive, anti-diabetic, anticarcinogenesis, antispasmodic and hypotensive, relaxant effect, neuroprotective and molluscidical from this ASF [27]. ASF consists of three main fractions; including resin (40-64%), gum (25%) and essential oil (10-17%) [28]. The resin fraction contains coumarins, sesquiterpene coumarins, ferulic acid and its esters and other terpenoids. The gum includes glucose, galactose, 1-arabinose, rhamnose, glucuronic acid, polysaccharides and glycoproteins, and the volatile fraction contains sulfur-containing compounds, monoterpenes and other volatile terpenoids. Sulfur compounds in *F. asafoetida* resin show various biological activities and can be valuable in medicine [27].

Curcumin (CUR) [diferuloyl methane] is naturally occurring flavonoid compound found in the plant *Curcuma longa* which is used as food additives and have been shown to possess a wide range of biological and pharmacological activities such as anti-inflammatory, anticancer, antioxidant, antimicrobial, neuroprotective, cardioprotective and radio-protective effects [13]. CUR strongly inhibits proliferation of HT-29 and HCT-15 human colon cancer cell lines [29].

TNF, ASF and CUR have been linked with gut health by beneficial effects in gastrointestinal inflammatory disorders and protection from colon cancer. In this regards on the basis of their safety, nutritional value and bio-pharmacological importance our research group designed and developed a formulation to improve gut health. The best of our knowledge, no study has been reported so far in the available literature describing ASF and CUR jointly encapsulated with TNF using spray drying technique. In the present study reports for the first time the design of gut health product (GHP) formulation by encapsulating ASF and CUR onto TNF. The GHP was characterized by SEM with EDS, IR, XRD and DSC studies. The study was
also carried out to evaluate the anti-colitis effect of gut health product in dextran sulfate sodium (DSS) induced colitis in rats.

2. Materials and methods

2.1. Materials and chemicals

Turmeric residues (after extraction of the active chemical constituents), ASF oleoresin and CUR (95%) were collected from Plant Lipids (P) Ltd, Kerala, India. The turmeric residues were cleaned thoroughly with running tap water followed by soaking in double distilled water and dried. Quillaja saponin (Q-Naturale® 200) was purchased from Ingredion India Pvt Ltd, Mumbai, India. All the chemicals used in the present study viz. sodium chlorite, acetic acid, dextran sulfate sodium (DSS) (MW= 36,000-50,000), hexadecyltrimethylammonium bromide, hydrogen peroxide, O-dianisidine dihydrochloride and disodium hydrogen phosphate were analytical grade and purchased from Sigma Aldrich, Mumbai, India.

2.2. Physical parameters

2.2.1. Bulk density

Bulk density (g/mL) of TNF and GHP was determined by adding 10 g of sample into an empty 100 mL graduated cylinder and place the cylinder on a ring stand and adjust the ring clamp so that, when the base of the cylinder is raised to touch the ring, the bottom surface of the cylinder is exactly one inch from the base of the ring stand. The ratio of mass of the sample and the volume occupied in the cylinder determines the bulk density values [30].
2.2.2. Moisture content and hygroscopicity:

The moisture content was determined based on the AOAC method [31]. Triplicate samples of TNF and GHP (20 mg) were weighed and then dried in a vacuum oven at 70 °C. The drying and weighing processes were repeated until constant weight was obtained. Hygroscopicity analysis of TNF and GHP was determined about 1 g of powder was spread evenly on Petri dishes to allow for a high surface area between humid air and powder. Samples of each powder in the dishes were placed in desiccators under the conditions of 23 °C and 76% relative humidity using nitric acid solution. The get in weight of the samples was considerably lower after 90 min. Although hygroscopicity is based on the equilibrium moisture content, to compare hygroscopicities, the weight increase per gram of powder solids after being subjected to the atmosphere with relative humidity of 76% for 90 min was determined [32].

2.2.3. Degree of caking (DC):

The samples were placed in a drying oven at 70 °C. After cooling, the dried samples were weighed and transferred into a sieve of 500 mm size. The sieve was shaken for 5 min in a shaking apparatus. The weight of the powder remaining in the sieve was measured. The degree of caking was calculated as

\[ DC = \frac{a}{b} \times 100 \]  (1)

Where DC is the degree of caking (%), ‘a’ is the amount of the powder used in sieving, and ‘b’ is the amount of the powder remained on the sieve after sieving [33].
2.3. Analysis and characterization of TNF and GHP

Analysis of the bioactive components present in the GHP was performed using ultra performance liquid chromatography (UPLC) (Shimadzu SP 20 AD, Nexera, Japan) for curcumin using column Xterra MS C18, 5µm, with volume 4.6 × 250 mm (Waters, USA). The mobile phase consisted of 40% tetrahydrofuran and 60% water with 1% citric acid. The detection was carried out in an isocratic flow at a wavelength of 420 nm using a photodiode array (PDA) detector. The elution was carried out by isocratic method with 1 mL/min flow rate. The volatile oil was hydro-distilled from GHP for 6 h using Clevenger apparatus and dried over anhydrous sodium sulfate and stored in a refrigerator (8 ± 2 °C). The volatile oil was subjected to gas chromatography-mass spectrometry (GC-MS) analysis using Brucker 436-GC, couple with SCION TQMS by Rtx-624 fused silica column with 60 m column length and 0.25 mm internal diameter. The column temperature was maintained initially at 50 °C and gradually increased to a rate of 10 °C per minute till to attain 230 °C. Helium gas was used as a carrier gas at 1 mL/min of flow rate with constant pressure as 228 kPa. The result of GC has interpreted using National Institute of Standard Technology - MS search library Version 2.0.

Fourier transform infrared (FT-IR) spectra of TNF and GHP were recorded by JASCO FT/IR-460 plus instrument in the range of 400 – 4000 cm⁻¹ with 32 scans per sample using the grounded and blended samples with KBr in a ratio of sample/KBr at 1:4. The surface morphology of the TNF, before and after encapsulation of bioactive compounds was determined using scanning electron microscopy (SEM). Elemental analysis was carried out by using Energy-dispersive X-ray spectroscopy (EDS) unit attached with SEM (Vega3Tescan, Brucker). Samples were placed in aluminum stubs using double-sided carbon tape and sputter coated with a fine
layer of gold using a sputter gold coater. All samples were examined using an accelerating voltage of 30 kV. The crystalline nature of the TNF before and after encapsulation of bioactive compounds were determined using X-ray diffraction pattern (XRD) (XRD-Xpert-Pro) at ambient temperature using Cu Kα radiation (λ = 0.1541 nm) over the 20 range of 10 and 80° with a scanning speed of 1.2°/min. All samples were vacuum dried at 60 °C before the assay. The thermal stability behavior of TNF and GHP was evaluated using differential scanning calorimeter (DSC) Q10 DSC instrument (Mettler Toledo DSC822e, India).

2.4. Anti-colitis activity study

2.4.1. Animals

Healthy adult Wistar albino rats of either sex weighing 100-150 g were used. The animals were acquired from the Acharya & B.M. Reddy college of pharmacy, Bangalore, India. Animals were housed in groups of 6 rats (3 male and 3 female) per cage by cage card and corresponding color body making in the experimental room at one week period. The animals were housed in polypropylene cages with stainless steel grill top under air conditioned rooms with optimal air changes per hour, relative humidity, temperature and elimination cycle set to 12 hours light and 12 hours in the dark. Amrut brand pellet feed and purified filter water were provided *ad libitum*. The protocol of anti-colitis activity was approved by the Institutional Animal Ethical Committee (IAEC) of Acharya & B.M. Reddy college of pharmacy (Reg. No. 997/c/06/CPCSEA), Bangalore, India and conducted according to the guidelines of Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).
2.4.2. Treatment groups

The animals were randomly divided into 3 groups, containing six animals in each. Group 1 served as a healthy control which receive water only and maintained on regular rat food and drinking water *ad libitum*. Remaining two groups (Group 2 – colitis control and Group 3 – GHP control) were received freshly prepared 5% DSS solution in water for 6 days and animals were provided with food and water. Group 3 was received GHP (400 mg/kg) for 21 days after the induction with DSS [34]. All rats were sacrificed by cervical dislocation and their colon tissues were isolated for the analysis of colitis score, myeloperoxidase (MPO) and histopathology on day 22.

2.4.3. Evaluation of clinical colitis

Rats were scored daily with respect to body weight, stool formation and fecal occult blood. Disease activity index (DAI) was determined by average of combined score of weight loss, stool formation and fecal occult blood. Scores were assigned as follows: weight change (0: <1%, 1: 1–5%, 2: 5–10%, 3: 10–15%, or 4: >15%), fecal occult blood (0: negative, 2: positive, or 4: gross bleeding), and stool formation (0: normal, 2: loose stool, or 4: diarrhea). Scoring was performed as previously described by Cooper et al [35] and Sasaki et al [36].

2.4.4. Assessment of colitis score

After washing the mucosa with saline (0.9%) solution, the macroscopic appearance of the colonic mucosa was scored by an independent observer according to a scale ranging from 0 to 3 as follows: 0 for no ulcer; 1 for inflammation, edema, thickness, and superficial ulcer; 2 for bleeding and deep ulcer; and 3 for necrosis and/or perforation. After macroscopic evaluations,
the tissues were cut into three equal parts. One part of the sample of colonic tissue was preserved in 10% formalin for histologic examination and the other two colonic samples were frozen in liquid nitrogen and stored immediately at –70 ºC until analysis [37].

2.4.5. Myeloperoxidase activity (MPO) on colon tissue

The colon tissue was homogenized in phosphate buffer containing 0.5% hexadecyl trimethylammonium bromide using a polytron homogenizer to produce a 10 % w/v homogenate. After freeze-thawing for three times, the samples were centrifuged at 15000 rpm for 30 min at 40 ºC and the resulting supernatant assayed spectrophotometrically for MPO. To 40 ml of the sample, 960 ml of phosphate buffer containing O-dianisidine dihydrochloride and hydrogen peroxide was mixed and shaken vigorously. The change in the absorbance was measured at 460 nm for 3 min at an interval of 60 seconds. The enzyme activity for one unit was defined as the quantity of MPO that causes an alteration in absorbance measured at 460 nm for 3 minutes. Myeloperoxidase activity was expressed as U/g tissue [38].

2.4.6. Histopathology analysis

The rats were sacrificed by cervical dislocation and kidney was isolated, these organs were quickly excised and perfused with chilled normal saline to completely remove all the blood cells and subjected to histopathological examination. A fresh piece of the kidney from each rat, previously trimmed to approximately 2 mm thickness, was rapidly fixed at 10% neutral formalin. The fixed tissues were then embedded in paraffin, sectioned (5 µm) with a rotary microtome and stained with haematoxylin and eosin (H&E). The kidney sections were evaluated histologically with a camera attached to a light microscope (Nikon E400).
2.5. Statistical analysis

Data were expressed as mean ± standard error of mean. Statistical comparisons were made by using one-way ANOVA followed by the Dunnet multiple comparison test. The results were considered statistically significant at P < 0.01.

3. Results and discussion

3.1. Preparation of gut health product (GHP)

3.1.1. Preparation of turmeric nanofiber (TNF)

The cleaned turmeric residues were bleached with aqueous sodium chlorite (1.7 wt % NaClO$_2$ in water) at 90 °C for 2 h after that acetic acid was added to attain pH 4 to enhance the bleaching action. The resultant turmeric dietary fiber was washed repeatedly with distilled water until the pH of the effluent became neutral and subsequently dried at 50 °C for 12 h in an air circulating oven [39]. The turmeric dietary fibers were grounded and treated with 4 % sodium hydroxide solution (w/w) at 80 °C for 2 h under mechanical stirring which partially solubilized pectin, lignin and hemicelluloses (Alkali treatment). This treatment was done at least 3 times, in order to purify cellulose. After each treatment fibers were filtered and washed with distilled water until the alkali was completely removed, checked by pH meter. A successive bleaching action was carried out to bleach the fibers. The solution used for this treatment was made of equal parts of acetate buffer, aqueous chlorite (1.7% (w/w) in water) and distilled water (Bleaching treatment). This bleaching treatment was performed at 80 °C about three times under mechanical stirring. After each treatment the fibers were filtered and washed with distilled water, which resulted in further effective discoloration and confirming the leaching out of phenolic
compounds and lignin. Acid hydrolysis was achieved by subjecting 6–8% (w/w) bleached fiber at 50 °C with 65% (w/w) sulfuric acid for about 60 min, using mechanical stirring (Acid hydrolysis treatment). The acid hydrolysis treatment helped in leaching out traces of minerals, residual starch and also hydrolyzed amorphous cellulose and eased in getting the required nanofibers. After each step of chemical treatment, the suspension was diluted with ice cubes to stop the reaction and washed with distilled water and centrifuged at 10000 rpm at 10 °C for 20 min. The resultant turmeric nanofiber (TNF) was homogenized by using a PRIMIX-Homomixer Mark II-2.5 homogenizer for 10 min and filtered using glass filter. The TNF was powdered and used for encapsulation of bioactive molecules.

3.1.2. Encapsulation of bioactive compounds with TNF

The unique product GHP made by the following steps which involved, TNF was added to the commercial Q-Naturale® ingredient previously dissolved in water and an emulsion was obtained in a homogenizer at 8000 rpm for 10 min. The bioactive compounds 95% CUR (2%) was initially added to the emulsified solution followed by ASF oleoresin (1%) were added and vigorously homogenized for 30 min at 80 °C temperature. The emulsion was dried by the spray drying process. The resultant product is GHP.

3.2. Physical parameters

3.2.1. Bulk density

The bulk density is a significant characteristic of powder/granule products in term of storage and simplicity of transportation. GHP registered considerably lower bulk density value (0.38 mg/mL) than TNF (0.55 mg/mL) (Table 1) because of high drying temperature was used
during the spray drying process of preparation of GHP, but TNF was prepared by powdering process. At high temperatures, very high drying processes are achieved implying a lower shrinkage of the droplets and consequently a lower density of the powder [40].

3.2.2. Moisture content and hygroscopicity

The moisture content of TNF and GHP was found 2.1% and 0.9% respectively (Table 1). GHP was registered lower moisture content rather than the TNF because the processes of preparation under spray drying technique. Generally, the process spray drying technique, increasing drying temperature resulted in greater loss of water of resultant product, due to the higher rate of heat transfer into particles, causing faster water removal. This is shown in the result of moisture content of GHP reduced quickly due to the processing inlet temperature at 180 °C. At high temperature, as evaporation rate is faster, GHP dry to a more porous and fragmented structure and implying a lower shrinkage of the droplets, and so the lower density of the product [40,41]. The lower hygroscopicity result of spray dried product GHP (0.11 g/g) rather than the powdered product of TNF (0.29 g/g) is also well agreement with this result due increase in let temperature of the spray drying process (Table 1).

3.2.3. Degree of caking

Degree of caking was lower in the case of spray dried product GHP (2.54%) rather than the powdered product TNF (4.28%) (Table 1). Degree of caking is a direct relationship with moisture content and hygroscopic nature of the product. The degree of caking in the case of GHP comparatively decreased with an increase in inlet air temperature during the spray drying process [32].
3.3. Chemical analysis and characterization

3.3.1. Chemical analysis

The chemical analysis of GHP by UPLC and GC-MS was done for the chemical profile of GHP. The presence of CUR was confirmed by UPLC analysis and the chromatogram is shown in Fig. 1 (a). Volatile oil was obtained from GHP by hydrodistillation was analyzed by GC-MS. Chromatogram and identified compounds were present in the GHP were given in Fig. 1 (b). The major compounds were 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, n-propyl sec-butyl disulfide, 1,2-dithiolane, disulfide bis(1-methylpropyl), 1,3-dithiane, 2,2-bis(methylthio) propane and 1-(1-propen-1-yl)-2-(2-thiophen-3-yl) disulfide. The analysis clearly confirmed that the GHP is formulated by encapsulation of CUR and sulfur containing ASF onto TNF.

3.3.2. Fourier Transform Infrared Spectroscopy (FT-IR) studies

The FT-IR spectra of TNF from turmeric spent and GHP (ASF and CUR encapsulated TNF) are elucidated in Fig. 2. In both the spectra, in the region of 3010-3653 cm\(^{-1}\), the broadened absorption band distinctive to the -OH stretching was observed both spectra which were attributed to O-H stretching of hydrogen bound to the hydroxyl groups originating mainly from cellulose and hemicelluloses [42]. The bands at 2854 and 2923 cm\(^{-1}\) were originated from C-H stretching vibrations, which represented the presence of the typical structure of polysaccharide compounds [43,44]. The prominent band of C-O-C pyranose ring skeletal vibration stretching around 1020 cm\(^{-1}\) was observed in both spectra and indicate the presence of xylans and is associated with hemi-celluloses which confirmed that xyloglucans are strongly bound to TNF and also the intense band at this wavenumber proves high cellulose content in
GHP [45,46]. The peak at 1633 cm\(^{-1}\) was corresponded to bending or stretching aromatic hydrocarbons of lignin, which was one more main component of dietary nanofibers [44,47]. In addition, the absorption band at 1155 cm\(^{-1}\) is contributed to the stretching of C-O groups of C-O-C aliphatic esters [48]. In the spectrum of GHP (Fig. 2 (b), the band at 1428 cm\(^{-1}\) is indicated the olefin bending vibration of the C=C group bound to the benzene ring which is present in the CUR [49]. The spectrum of GHP (Fig. 2 (b)) had weak to medium intensity bands in the region 626 to 730 cm\(^{-1}\) were due to the asymmetric and symmetric C-S stretching vibration due to the presence of more number of sulfur containing compounds which are present in the ASF. Moreover, bands with weak stretching in the range of 405 to 529 cm\(^{-1}\) indicative of stretching vibration of S-S linkage of sulfur compounds in the ASF. In addition the bands show multiplicity, probably due to the vibrational coupling of presence of tri and tetra sulfides. Two more weak bands were observed in the GHP spectrum at 751 and 900 cm\(^{-1}\) due to the stretching frequency of S-O linkage of compounds [50]. These data are clear evident for the encapsulation of the ASF with TNF. In the both spectra, there were no changes in the characteristic peaks on TNF exhibited any noticeable variations indicating the successful encapsulation of ASF and CUR onto DNF using homogenization and followed by spray drying process.

3.3.3. X-ray diffraction (XRD) studies

The XRD pattern of TNF and CUR encapsulated TNF and CUR and ASF encapsulated TNF (GHP) are shown in Fig. 3. These patterns clearly revealed two main peaks at \(2\theta = 16.4^\circ\) and \(2\theta = 21.2^\circ\), which represented typical cellulose I form indicating crystallinity of TNF [51]. A new peak appeared at \(2\theta = 26.1^\circ\) in the case of both CUR and CUR and ASF encapsulated TNF (GHP) in Fig. 3 (b) and (c), which is attributed to encapsulation of CUR onto TNF [52]. Fig. 3
(C) shows another new peak at $2\theta = 61.6^\circ$ in the XRD pattern of GHP (Fig. 3 (c)), which correspond to the encapsulation of sulfur compounds presented in the ASF [53].

3.3.4. Scanning electron microscopy (SEM) studies

The morphologies of the obtained TNF and GHP were studied using SEM. Fig. 4 shows the SEM images of the TNF, and CUR encapsulated TNF, and CUR with ASF encapsulated TNF (GHP). The micrographs confirm the successful encapsulation of the ASF and CUR with irregular spherical shape and very low agglomeration level. This is probably due to the very low percentage level of CUR and ASF only taken for the encapsulation in this process. The surface of the TNF is smooth by cluster like structure with the size range between 200 nm to 350 nm. Upon the encapsulation of CUR and ASF complex, there is no significant change in the size distribution indicating that encapsulation of the CUR and ASF did not negatively affect the encapsulation process.

3.3.5. Energy-dispersive X-ray spectroscopy (EDS) analysis

Encapsulation is further supported by EDS analysis (Fig. 5), which provides direct evidence for the encapsulation of ASF onto TNF by the presence of sulfur. The EDS mapping image is also used to explain the presence and distribution of ASF in the form of sulfur after encapsulation onto TNF (Fig. 5).The density of red spots for the sulfur as seen in the EDS image confirm that ASF encapsulation has occurred onto the TNF.

3.3.6. Differential scanning calorimeter (DSC) analysis

DSC is a useful technique to provide certain thermodynamic information, such as the temperature and enthalpy associated with transitions in materials during endothermic or
exothermic processes. Two endotherms with peak temperatures of the TNF (69.3 °C) and GHP (67.9 °C) were observed in the DSC thermograms in Fig. 6 are connected with water evaporation [54]. Interestingly, exothermic and endothermic processes did not occur until a temperature of 250 °C was reached, indicating that the TNF and GHP are highly thermally stable. Additionally, DSC is confirmed in the case of GHP, there is no further degradation of encapsulated ASF and CUR up to 250 °C. This also confirmed that ASF and CUR encapsulated onto TNF does not alter thermal degradation pattern.

3.4. Anti-colitis activity of GHP by dextran sulfate sodium (DDS) induced colitis in rats

Different drug treatments are currently used for the treatment of gut health, with the main goal of inducing and maintaining remission of symptoms and mucosal inflammation providing an improved quality of life to patients. Although they show efficacy in most of the cases, they are usually associated with the onset of different side effects that may strongly affect patient compliance and quality of life, thus limiting their use for prolonged periods of time. This has prompted the use of alternative and complementary treatments for gut health and, among these, the use of phytochemical formulations are continuously increasing [55]. Preparations of some phytochemical formulations may qualify for controlling biological studies based on their traditional use if well-described and reasonably expected to be safe in the target population, it is reasonable to develop a pharmacological rationale first, subsequently allowing as well for a targeted approach to appropriate quality assurance measures. With this purpose, the present study was focused on examining the anti-colitis activity of GHP - a standardized complete natural matrix contains TNF, CUR and ASF, very rich in polyphenols and sulfur containing compounds, in preventing or minimizing the effects of DSS-induced colitis in rat. The DSS model in rat
produces inflammation limited to the colonic mucosa that is more closely related to human ulcerative colitis [56]. The DSS induced-colitis animal model has a number of advantages over other models such as simple experimental methods, reproducibility of the development time-course as well as colitis severity among individual rat, and relative uniformity of the induced lesions. Therefore, this experimental model is reliable for studying the pathogenesis of UC and for testing drugs or phytochemicals for treatment [57,58]. We have demonstrated for the first time that administration of GHP effectively prevented colonic inflammation in rat. To evaluate the inhibitory effect of DSS-induced colitis by GHP in rat system, the body weight, DAI, colitis score, colonic MPO activity and histopathological study was detected and the DAI, colitis score and MPO activity significantly reduced.

3.4.1. Body weight change

The weekly interval body weight is shown in Fig. 7. The 21 days administration of GHP by oral to rats causes the slightest changes in the body weight, but statistically there is no significant changes compared to the colitis control on days 7 and 14. However, on final day of the study, there is statistically significant (p<0.05) changes of body weight compared to the colitis control. Treatment with DSS led to severe loss viz. -1.26% on day 7, -3.81% on day 14 and -7.23% on day 21 of body weight compared with initial days of the study. However, treatment of DSS induced rats with GHP initially decreased (-3.85% on day 7) and significantly increased the body weight 5.42 and 6.88% (P<0.05) on days 14 and 21 respectively with respect to the initial day of the study (Fig. 7).
3.4.2. Disease activity index score and colitis score

Disease activity index (DAI) (Fig. 8) and colitis scores (Fig. 9) were determined for the DSS control group. The scores resulted in considerable increases with the score values compared to the score values of healthy control. In the case of DAI, the score values are increased for DSS control groups and the values are 7.75 ± 0.47, 11.50 ± 0.64 and 13.25 ± 0.62 on days 7, 14 and 21 respectively compared to healthy controls with a score value of one. Colitis score also registered higher value (2.25 ± 0.25) for the DSS control group on day 21. Treatment with GHP suppressed significantly (P<0.01) the DSS induced increases in DAI (5.75 ± 0.25, 5.25 ± 0.47 and 2.75 ± 0.25 on days 7, 14 and 21 respectively) and colitis score (1.0 ± 0.01).

3.4.3. Myeloperoxidase activity (MPO) on colon tissue

Colonic levels of MPO in selected study groups were assessed and compared with the levels in control groups. The level of colonic MPO activity was dramatically increased in DSS-induced colitis control rat (8.76 unit/100 mg of wet tissue), and that GHP markedly reduced the MPO activity (3.24 unit/100 mg of wet tissue) (Fig. 10) suggesting an anti-inflammatory effect of GHP.

3.4.4. Histopathological studies

The microscopic analysis of the colonic samples revealed the beneficial effect exerted by the GHP of experimental colitis. Fig. 11 shows the haemotoxylin and eosin (H&E) staining of colons of healthy control, DSS induced colitis control and GHP control. Photomicrographs were subsequently obtained at 50 and 200× magnifications. The healthy control group showed normal mucosa by the appearance of blood vessels in sub-mucosa with intact epithelial containing
adequate goblet cells (Fig. 11 (a, b)), while DSS induced colitis control group showed partially ulcerated colonic mucosa with falling off glandular epithelial cells into the lumen (Fig.11 (c), yellow arrow). The epithelial cells contain decrease in goblet cells (Fig. 11 (d), yellow arrow) with dense mononuclear inflammatory infiltration (Fig. 11 (d), red Arrow). The sub-mucosa shows edema containing scattered inflammatory infiltration (Fig.11 (c), red arrow). The GHP control group showed intact colonic mucosa with mucin material secretion into the lumen (Fig.11 (e), red arrow). The epithelial cells contain adequate goblet cells (Fig. 11 (f), red arrow) with mild mononuclear inflammatory infiltration (Fig. 11 (f), yellow arrow). Thus, a significant reduction in the extension of colonic damage was observed in treated colitic rats with the GHP. The treatment with GHP showed a significant recovery of the intestinal histology compared to control group. The development of the inflammatory progression was observed mostly in submucosa and muscularis layers. In consequence, the fact that GHP has shown beneficial effects in DSS induced models of experimental colitis makes it a good candidate for gut health therapy without toxicity as a functional food.

In fact, compounds like curcumin from *C. longa* and sulfur containing compounds from ASF and DF from *C. longa* have proven antioxidant and anti-inflammatory activity by the various models [59], and DF isolated from *C. longa* has beneficial action by anticancer and anti-inflammatory activity against various disease like colon cancer, diabetics and heart diseases due to its action in the gastrointestinal tract as insoluble matrix [14,15]. ASF and CUR with few common spices or their active principles such as capsaicin, piperine, ginger, cumin, fenugreek and mustard were examined for their possible influence on digestive enzymes of the pancreas in experimental rat. Among these spices, ASF and CUR prominently enhanced pancreatic lipase
activity and also stimulated pancreatic amylase. The positive influence of the pancreatic digestive enzymes exerted by consumption of ASF and CUR could be a factor contributing to the well recognized digestive stimulant action of spices [60]. The same research group also examined the in vitro influence of fourteen spices with ASF and CUR on the activities of digestive enzymes of rat pancreas and small intestine by including them in the reaction blend at two dissimilar concentrations. ASF and CUR enhanced the activity of pancreatic lipase and amylase when they are directly in contact with the enzyme. It is inferred that this positive influence on the activity of enzymes may have a supplementary role in the overall digestive stimulant action of ASF and CUR, besides causing an enhancement of the titers of digestive enzymes in pancreatic tissue [61]. The relaxant effect of ASF and essential oil from ASF seed was investigated in isolated ileum of rat after three doses. ASF produced an antispasmodic effect on acetylcholine (Ach) induced contraction in 0.2% and 0.3%. Spasmolytic evaluation showed that the essential oil derived from F. asafoetida seed in concentrations of 0.2 and 0.3%, significantly reduced Ach from 10 to 4 M induced concentrations. Exposure to the 0.2 and 0.3% ASF, reduced statistically significant, the percentage of maximum contraction induced by 10 to 4 M Ach to 43% and 12% respectively. ASF can be used as an antispasmodic therapeutic agent [62]. The result of ASF on muscarinic receptors of tracheal soft muscle was investigated for relaxant effect. The effects of three cumulative concentrations of aqueous extract of F. asafoetida (2.2, 5 and 10 mg/mL), 10 nM atropine and saline on muscarinic receptors were tested on tracheal smooth muscle samples. The maximum responses to methacholine in the presence of higher concentration of the ASF (10 mg/mL) were significantly lower than that of saline. Because of F. asafoetida or its constituents may bind to muscarinic receptor of tracheal smooth
muscle and put off the binding of methacholine to this receptor, it suggested the competitive antagonistic effect of *F. asafoetida* at muscarinic receptors.

The effects of CUR on the development of DSS-induced experimental colitis were evaluated with BALB/c mice were fed a chow containing either 3.5% DSS or 3.5% DSS + 2.0% CUR. The body weight loss was more apparent in DSS-treated group than in DSS + CUR-treated mice. The disease activity index, histological colitis score, and MPO activity were all significantly higher in DSS-treated group than in DSS with CUR treated group. Microscopically, mucosal edema, cellular in- filtration, and epithelial disruption were much more severe in DSS-treated group than in DSS with CUR-treated group the development of DSS-induced colitis was significantly attenuated by curcumin. Being a nontoxic, natural dietary product, curcumin could be useful in treatment of IBD patients [63]. The preventive effects of curcumin were investigated by Kao et al [64] using DSS-induced colitis and the potential role of curcumin in regulation of anti-inflammation through S-nitrosylation. After curcumin treatment for 6 days, the body weight and disease activity index of DSS-induced mice were alleviated and the colonic length was also rescued. Moreover, Curcumin reduced the amount of nitrite in DSS-induced colitis but not affected total S-nitrosylation level of proteins on day 6, indicating that curcumin inhibited NO oxidation. Additionally, the safety of S-nitrosylation on IκB in DSS-induced colitis for 6 days by CUR caused the repression of IκB phosphorylation and NF-κB activation. The study revealed that curcumin-mediated S-nitrosylation may be as an important regulator for anti-inflammatory in DSS-induced colitis of mice [64]. The anti-inflammatory activity of CUR was evaluated in the form of co-encapsulated CUR with piperine on DSS-induced colitis model and the results showed that CUR exhibited definite anti-colitis activity in inflammatory colon tissue through
retention enema administration [65]. Eudragit S100 (ERS100)/PLGA microparticles (MPs) were fabricated with pH sensitivity nature for colitis-specific curcumin delivery. In vivo experiments demonstrated that curcumin fabricated microparticles exhibited a much better therapeutic efficacy against experimental ulcerative colitis (UC) compared with curcumin [66].

In this study ASF and CUR encapsulated with TNF as a complete natural matrix because of DF has been proven to be beneficial in maintaining remission in human ulcerative colitis. DF supplementation ameliorated colonic damage in HLA-B27 transgenic rats. This effect was associated with an increased production of short-chain fatty acids, which can act synergistically in inhibiting the production of pro-inflammatory mediators [67]. The body weight loss and disease activity index were significantly lower in a DF containing product treated mice compared with the glucose-treated mice. The decrease in colon length induced by DSS was significantly alleviated in DF containing product treated mice compared with glucose treated mice. The histologic findings showed that intestinal inflammation was significantly attenuated in mice treated with the DF containing product. Furthermore, treatment with a DF containing product significantly inhibited the dextran sulfate sodium–induced increase in the mRNA expression of interleukin-1β. It is suggested that DF has potential therapeutic value as an adjunct therapy for ulcerative colitis [68].

In this study, we evaluated the development of the disease for 21 days after received 5% DSS solution in water for 6 days. The highest DAI score was observed in the DSS group that developed intense weight loss, fecal bleeding and persistent and severe diarrhea. On the other hand, the animals treated with the GHP did not show the clinical symptoms of colitis or weight loss. Because UC is characterized by an intense inflammatory process, the ability of the GHP to
reduce the colitis clinical symptoms demonstrates the anti-inflammatory, antioxidant and anticancer activities of ASF and CUR. In addition, the colon histological analysis shows that the animals that received water with DSS presented typical inflammatory changes in their colon architecture that was slightly attenuated when the animals were treated with the GHP. These effects on clinical symptoms and on histological parameters could be due to the presence of anticancer, antioxidant and anti-inflammatory compounds, such as CUR has been isolated from C. longa [13] sulfur containing compounds from ASF [27]. This study demonstrates that ASF and CUR encapsulated with TNF formulation GHP, ameliorate the DSS-induced colitis in rats through the reduction in DAI and histopathological lesion, and downregulating inflammatory mediators such as MPO activity.

4. Conclusion

A novel gut health formulation GHP was prepared by encapsulation of ASF and CUR onto TNF through spray dry technology with complete natural matrix. The chemical profile of GHP was analyzed by UPLC and GC-MS and confirmed the presence of CUR and sulfurous compounds well encapsulated with TNF and it is further confirmed by EDS analysis. The SEM confirm the successful encapsulation of ASF and CUR onto smooth and cluster like nanostructured TNF. IR, XRD and DSC analysis further confirmed the presence of ASF and CUR in the GHP. GHP treatment effectively improved characteristic IBD symptoms and histological scores, attenuated inflammation and maintained intestinal integrity in DSS-induced UC. Based on the findings, GHP could serve as an effective treatment for amelioration of UC.
Conflict of interest

There are no conflicts of interest to declare

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References


proteins - A study on gluten dough with application of FT-Raman spectroscopy, TGA and DSC, Food Hydrocoll. 69 (2017) 410-421.


Fig. 1. UPLC chromatogram of curcumin in GHP (a) and GC-MS chromatogram of sulfurous compounds in GHP (b): (1) 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, (2) n-propyl sec-butyl disulfide, (3) 1,2-Dithiolane, (4) Disulfide, bis(1-methylpropyl), (5) 1,3-dithiane, (6) 2,2-bis(methylthio) propane and (7) 1-(1-propen-1-yl)-2-(2-thiophen-3-yl)disulfide
Fig. 2. FT-IR spectra of (a) TNF and (b) GHP
Fig. 3. XRD pattern of (a) TNF (b) CUR encapsulated TNF and (c) GHP
Fig. 4. SEM images of (a) TNF, (b) CUR encapsulated TNF and (c,d) GHP
Fig. 5. EDS spectrum and mapping image (insert) of GHP
Fig. 6. DSC thermogram of (a) TNF and (b) GHP
Fig. 7. Body weight changes of the rat at four different study intervals in health control, DSS-colitis control and GHP.
Fig. 8. Disease activity index of the rat at four different study intervals in health control, DSS-colitis control and GHP.
Fig. 9. Colitis score of the rat at four different study intervals in health control, DSS-colitis control and GHP
Fig. 10. MPO activity of the rat at four different study intervals in health control, DSS-colitis control and GHP.
Fig. 11. Histological sections of colonic tissue stained with haematoxylin and eosin showing the effects of healthy control (a,b), DSS-induced colitis control (c,d) and GHP control
Table 1
Physical properties of TNF and GHP

<table>
<thead>
<tr>
<th>Properties</th>
<th>TNF</th>
<th>GHP</th>
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<tbody>
<tr>
<td>Bulk density (g/mL)</td>
<td>0.55±0.07</td>
<td>0.38±0.06</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>2.1±0.8</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>Hygroscopicity (g/g)</td>
<td>0.29±0.05</td>
<td>0.11±0.04</td>
</tr>
<tr>
<td>Degree of caking (%)</td>
<td>4.28±1.2</td>
<td>2.54±0.59</td>
</tr>
</tbody>
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Values are means ± SD of three independent determinations
Graphical abstract
Research highlights

- Ulcerative colitis (UC) is a main form of inflammatory bowel disease (IBD)
- Gut health product (GHP) was formulated by encapsulation of ASF and CUR onto TNF
- GHP was characterized by UPLC, GC-MS, FTIR, XRD, SEM with EDS and DSC studies
- GHP was evaluated for anti-colitis activity in a rat model of 5% DSS induced UC
- GHP has significant protective effects against DSS induced colitis