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Full Length Article

Beyond the flavor: A green formulation of *Ferula asafoetida* oleo-gum-resin with fenugreek dietary fibre and its gut health potential

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ABSTRACT

Albeit the fact that asafotida is a popular kitchen spice and Indian folklore medicine for gut disorders, its consumption at physiologically relevant dosage is greatly challenged by the unpleasant flavor characteristics. Herein we report a green approach to derive stable powder formulations of asafoetida gum with minimized taste and odor suitable for dietary applications and gut health-related disorders. Employing a water based ultrasound mediated gel-phase dispersion of asafoetida gum on fenugreek derived soluble galactomannan fibre matrix. Microencapsulated particles (1 ± 0.3 μm) of asafoetida was prepared as water dispersible free flowing powder (Asafin). Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), accelerated stability and *in vitro* dissolution studies confirmed the stability, sustained release and microencapsulated structure of Asafin. Further investigations revealed significant ($p < 0.01$) reduction in acetic acid-induced writings and inhibition of ethanol-induced ulcer (94.1%) in rats orally administered with Asafin at 250 mg kg^{-1} b.w. Asafin also exhibited anti-inflammatory effects ($p < 0.01$), in acute and chronic paw edema mice models. The safety of Asafin was further demonstrated by acute toxicity studies at 4 g kg^{-1} b.w. and by 28 days of sub-acute toxicity studies at 2.0 g kg^{-1} b.w.

1. Introduction

The incidence of gastrointestinal ulcers, a chronic degenerative disease which disrupts the mucosal integrity of the stomach and/or duodenum leading to a local defect and active inflammation, was found to be growing globally [1,2]. Ulcerative colitis (UC) and peptic ulcer (PU) are the most common ulcers of the gastrointestinal (GI) tract [3]. UC is an inflammatory bowel disease (IBD) characterized by prolonged inflammation of the digestive tract and was found to mainly affect the colonic mucosa [4]. Though the exact causes of UC are still not clear, different etiologic factors such as hereditary, infections, smoking, alcoholism, and medications were very often regarded as the main causative principles [5]. Peptic ulcer on the other hand is a broad term generally using for the ulcers in the stomach and duodenum, which reported to develop upon an imbalance between the 'aggressive' and 'protective' factors at the luminal surface of the epithelial cells [6]. While *helicobacter pylori*, hydrochloric acid, pepsins, non-steroidal anti-inflammatory drugs (NSAIDs), bile acids, ischemia, hypoxia, smoking and alcohol were identified as the aggressive factors in the pathophysiology of ulcers [7], defensive factors included bicarbonate, mucus layer, mucosal blood flow, prostaglandins and growth factors [8]. In sum, the pathogenesis of gastric ulcers generally include mucus layer depletion, chronic inflammation, and generation of free radicals with significant effect upon the quality of life [2,9].

Peptic ulcer was identified as the major gastrointestinal disorder affecting almost 10% of world population [10,11]. Though the antacids and antiulcer drugs constitute significant pharmaceuticals market share, the practice of herbal medicines and supplements over synthetic drugs continues to be the first choice for gut health globally [10]. The strong feeling of safety, relatively low incidence of side effects, cultural acceptability and cost effectiveness serves as the basic reasons for the attraction towards naturally alternative medicinal approach [12]. Yet another growing area of interest is to derive novel food components of medicinal values from GRAS (Generally Recognized as Safe)-listed spices, vegetables and fruits. The present contribution describes gut health related pharmacological effects of a novel formulation of asafoetida gum (*hereinafter referred to as 'Asafin'*) using the soluble dietary fibre isolated from fenugreek seeds. Fenugreek soluble fibre was characterized as galactomannans and has been clinically shown to possess hypoglycemic, hypolipidemic and gastroprotective effects [13,14].

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Earlier we had reported enhanced bioavailable formulations of curcumin using fenugreek galactomannans [15].

Asafoetida, the oleo-gum-resin of the herbaceous plant *Ferula asafoetida* root exudate, is an ancient condiment and a medicine in Europe and the East [12]. It is a pungent and bitter spice and a popular household remedy in India for stomach bloat, flatulence and digestive problems [12,16,17]. Though many recent pharmacological studies have demonstrated the antioxidant [18], anti-diabetic [19], laxative [12], anticancer [20,21], antiviral [22] and antifungal [23] effects of oleo-gum-resin of asafoetida, no stable formulations suitable for human supplementation have so far been reported mainly due to the apparent difficulty associated with the characteristic sulfurous odour, taste and gummy nature of asafoetida gum. High level of volatile oil content [10 to 20% (w/v)] rich in sulfur compounds was recognized as the reason for the unpleasant characteristics of asafoetida oleo-gum-resin.

2. Materials and methods

2.1. General

The proprietary formulation of asafoetida gum-resin (patent pending and registered formulation as 'Asafin™') was obtained from M/S Akay Flavours & Aromatics Ltd, Cochin, India along with a detailed certificate of analysis indicating its contents of asafoetida gum, dietary fibre, volatile oil, ferulic acid and other safety requirements as per US Pharmacopeia [24] (USP) ⟨561⟩, including pesticides, microbial counts, mycotoxins and heavy metals (2014). Asafin sample was prepared from the gum resin collected from Iran and was identified by an authenticated botanist. A voucher specimen (AK-ASF-01) was deposited at the Herbarium of M/s Akay Flavours & Aromatics Ltd, Cochin, India. Volatile oil content was measured using modified Clevenger method, standardized and approved (method 5.0) by the official methods of analysis, American Spice Trade Association [25]. Ferulic acid standard was obtained from Sigma-Aldrich, Bangalore, India and estimated by an HPLC procedure employing Shimadzu model LC 20 AT, with an M20A photo diode array (PDA) detector (Shimadzu Analytical India Pvt Ltd, Mumbai, India), fitted with a reverse phase C18 column (250 × 4.6 mm, 3 μm) (Phenomenex, Hyderabad, India). Water with 10% acetic acid (A) and acetonitrile with 20% acetic acid was employed as the mobile phase and monitored at 319 nm. Fourier-transform infrared spectra (FTIR) was recorded on Shimadzu 8700 spectrophotometer using potassium bromide (KBr) pellets prepared by compressing the powder at 20 psi for 10 min (Shimadzu Analytical Pvt. Ltd., Mumbai, India). The spectra were scanned over the wave number range of 3600–400 cm^{-1}. Scanning electron micrograph was performed on SEM Jeol 6390 LA equipment (JEOL Ltd., Tokyo, Japan).

2.2. Stability studies

Guidelines of the International Conference on Harmonization (ICH) were followed for stability studies [26]. Briefly, 10 g Asafin samples were packed in double layered polyethylene bags and kept in high density polyethylene bottles under air-tight conditions. The samples were incubated at 40 °C ± 2 °C and 65 ± 5% relative humidity for a period of six months and were analysed every month for various physicochemical parameters (colour, odour, volatile oil, fibre and ferulic acid contents).

2.3. Animals

Wistar rats (aged 4–5 weeks) of 150–200 g body weight were used for toxicity and anti-ulcer studies and male Swiss albino mice (aged 6 weeks) of 25 ± 5 g body weight were employed for anti-inflammatory and anti-nociceptive studies. Animal experiments were in accordance with the protocol approved by the Institutional Animal Ethics Committee, recognized by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (Registration No:149/99/CPCSEA). Animals were procured from Veterinary College, Kerala, India and were provided with pellet diet and water *ad libitum*. All animals were housed at the animal house facility of M/s Amala Cancer Research Centre, Kerala, India, in an air-conditioned room at 22 ± 2 °C and relative humidity 60 ± 5% with 12 h light and dark cycle.

2.4. In vitro antioxidant effect

The *in vitro* antioxidant effect and radical scavenging capacity of Asafin were investigated using standardized assays. Superoxide radical scavenging activity of Asafin was estimated by following the method of McCord and Fridovich [27], through measuring the reduction of nitro blue tetrazolium salt (NBT) by superoxide radicals generated during the photo reduction of riboflavin [27]. The hydroxyl radical scavenging activity was measured by studying the competition between deoxy ribose and Asafin for the hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radicals attack deoxyribose, which eventually results in the formation of thiobarbituric acid reactive substances [28,29]. The free radical scavenging activities were determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method reported by Brand-Williams et al. [30]. The level of lipid peroxidation in rat liver homogenate (10%) was measured using the method of Ohkawa et al. [29] by measuring the complex formed by the major secondary product of lipid peroxidation, malondialdehyde (MDA) and thiobarbituric acid (TBA).

2.5. Toxicity studies

2.5.1. LD_{50}

Adult Wistar rats of both sexes were randomized into four groups in such a way to have three male and three female rats per each group. Normal control group (I) was provided with 1 mL water and Groups II, III and IV were administered with Asafin at doses of 1, 2 and 4 g kg^{-1} b.w., respectively. All the animals were observed for 24 h for any adverse reactions or mortality and further continued for 14 days.

2.5.2. Repeated dose toxicity study (28 days)

Wistar rats of both sexes (20 males and 20 females) were randomized into four groups each consisting of five males and five females of approximately the same body weight and treated as follows. Group I – Normal (1 mL water); Group II – Asafin (2 g kg^{-1} b.w.); Group III – Asafin (1 g kg^{-1} b.w.); Group IV – Asafin (0.5 g kg^{-1} b.w.). The animals were monitored during the study period of 28 days for any type of clinical symptoms, mortality, and/or adverse reactions. Body weight was measured on every three days. Food consumption was determined on a daily basis and expressed as average of five animals per cage. On day 29, animals were sacrificed by cervical dislocation under ether anesthesia. Blood was collected into heparinized and non-heparinized tubes for both haematological and serum biochemical parameters. Serum was separated by centrifugation at 6000 rpm for 10 min at −4 °C and was stored at −20 °C. Total protein, albumin, bilirubin, cholesterol, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), serum urea and creatinine were measured by standard laboratory procedures. A haematology analyzer (Model-Diatron, Wein, Austria) was employed for the measurement of Red blood cell (RBC) count, total and differential white blood cell (WBC) count, platelet levels and haemoglobin (Hb) content. All the organs (liver, kidney, brain and spleen) were visibly examined for any type of abnormalities, excised, extraneous tissues were trimmed off and the weight was noted.

2.6. Anti-inflammatory activity

2.6.1. Carrageenan induced acute paw edema model

Male Swiss albino mice of body weight 25 ± 5 g were divided into four groups comprising six animals per group. The paw edema (acute inflammation) was induced according to [31], by a sub-plantar injection of 0.02 mL of 1% (w/v) of carrageenan solution at the right hind paw of the rat. Group I was injected with carrageenan alone; Group II was the positive control administered with the standard reference drug, diclofenac at 10 mg kg^{-1} b.w. *i.p.* Groups III and IV were orally administered with 250 and 500 mg kg^{-1} b.w. of Asafin, respectively for seven consecutive days. An hour after the seventh dose of Asafin, acute inflammation was induced. The inflammation was then measured using Vernier calipers one hour before (0 h) and at different post-injection intervals (1, 2, 3, 4, 5, 6 and 24 h). The percent of inhibition was then calculated as follows.

$$\%\text{inhibation} = \frac{[(V_T - V_0)_{\text{control}} - (V_T - V_0)_{\text{treated}}] \times 100}{(V_T - V_0)_{\text{control}}} \quad (1)$$

where, V_T – paw edema at various time intervals and V_0 – initial paw edema.

2.6.2. Formalin induced chronic paw edema model

Male Swiss albino mice of body weight 25 ± 5 g were divided into four groups, each comprising six animals. Group I was kept as formalin control and Group II was treated with diclofenac (10 mg kg^{-1} b.w., *i.p*). Group III and IV were orally treated with 250 and 500 mg kg^{-1} b.w. of Asafin respectively. Chronic inflammation was induced by sub-plantar injection of freshly prepared 0.02 mL of 2% formalin on the right hind paw in all groups [32]. Drug treatment was started one hour prior to formalin injection and continued for six consecutive days. The inflammation was measured using Vernier calipers before and after injection of formalin and continued for six consecutive days. The percentage inhibition was calculated using the same formula as given in Section 2.6.1.

2.7. Anti-nociceptive activity

Male Swiss albino mice of body weight 25 ± 5 g were divided into four groups with six animals per group. Acetic acid [0.6% (v/v), 10 mL kg^{-1} b.w.] was injected into the peritoneal cavities of mice, which were placed in a large observation boxes, and the intensity of nociceptive behavior was quantified by counting the total number of writhings occurring between 0 and 20 min after stimulus injection [33]. Group I was kept as acetic acid control and group II received the standard reference drug, aspirin (100 mg kg^{-1} b.w., *i.p.*). Group III and IV received Asafin orally at doses 250 and 500 mg kg^{-1} b.w. respectively. The drug treatments were given 30 min prior to acetic acid injection. The writhing response consisted of a contraction of the abdominal muscle together with a stretching of the hind limbs. The antinociceptive activity was expressed as the writhing scores over a period of 20 min. Antinociceptive effect was expressed as reduction of number of writhes between control and treated groups, using the formula:

$$\text{Reduction in writhes} = \left(\frac{(C - D)}{C}\right) \times 100 \quad (2)$$

Where, C is the average number of writhing's for control group of mice and D is the average number of writhing's of the drug treated mice.

2.8. Antiulcer activity

2.8.1. Effect of pretreatment of 'Asafin'

Thirty male Wistar rats were divided into six groups as follows.

Group I – Normal (1 mL water)
Group II – Control (80% ethanol, 5 mL kg^{-1} b.w.)
Group III – Ranitidine (50 mg kg^{-1} b.w.) + 80% ethanol (5 mL kg^{-1} b.w.)
Group IV – Asafin (50 mg kg^{-1} b.w.) + 80% ethanol (5 mL kg^{-1} b.w.)
Group V – Asafin (100 mg kg^{-1} b.w.) + 80% ethanol (5 mL kg^{-1} b.w.)
Group VI – Asafin (250 mg kg^{-1} b.w.) + 80% ethanol (5 mL kg^{-1} b.w.)

The animals were starved for 24 h and again deprived of both food and water for another 12 h prior to ethanol administration. Each group of animals were then treated with respective drug or vehicle as mentioned above. After 1 h of drug treatment, each group of animals was provided with ethanol at 5 mL kg^{-1} b.w. intragastrically to induce ulceration. After 3 h of ethanol administration, the animals were sacrificed by cervical dislocation, and the stomach of each animal was excised and washed with ice-cold saline. The ulcer index was then calculated by measuring the severity of the gastric mucosal lesion graded as Erosion grade 1: ≤1 mm mucosal lesion, Erosion grade 2:2 mm mucosal lesion, Erosion grade 3: ≥2 mm mucosal lesion [34].

$$\text{Ulser Index} = 1 \times [\text{no: of lesions of grade 1}] + 2 \times [\text{no: of lesions of grade 2}] + 3 \times [\text{no: of lesions of grade 3}] \times 10 \quad (3)$$

The gastric mucosal content was determined by the method of Corne et al. [35]. employing a standard curve of Alcian blue at 598 nm and the result was expressed in μg of Alcian blue per g of wet tissue.

2.8.2. Effect of post-treatment of 'Asafin'

Thirty male Wistar rats were divided into six groups and treated with various doses of Asafin and the standard drug Ranitidine as shown above. In this study, Asafin and ranitidine were supplemented after 1 h following the administration of 80% (v/v) of ethanol at 5 mL Kg-1 b.w. as described in Section 2.8.1 Groups IV, V and VI, were administered with Asafin at 50, 100 and 250 mg kg^{-1} b.w., respectively, after 1 h of ethanol administration. All the animals were sacrificed after 3 h and the stomach of rats were excised and washed with ice-cold saline. The ulcer index and gastric mucosal content were also investigated as in Section 2.8.1.

2.9. Statistical analysis

The values are expressed as mean ± SD and $p < 0.05$ was considered as statistically significant. Statistical analyses were performed using one-way analysis of variance (ANOVA) with the Dunnett multiple comparison test using Graph pad InStat software (version 3.05) of Graph Pad Software Inc, San Diego, CA, USA. The data for Asafin treated animals were compared with those for untreated animals.

3. Results

Iranian asafoetida oleo-gum-resin was used for the preparation of Asafin. Gel-phase dispersion followed by microencapsulation of asafoetida oleo-gum-resin on fenugreek soluble fibre matrix provided water dispersible free flowing granules of Asafin with particle size of around 1 ± 0.3 μm, as evident from the SEM (Fig. 1). Impregnation of asafoetida gum in the fibre matrix was further evident from the FTIR studies. The characteristic FTIR peaks of asafoetida gum was observed as sharp peaks at 1729.1, 1612.5, 1514.7 cm^{-1} were found to be present in Asafin along with the characteristic peaks of fibre at 1416.2, 1232.1, 1075, and 873.6 cm^{-1} respectively (Fig. 1A). Asafin showed minimized organoleptic properties and water solubility due to the effective microencapsulation of asafoetida gum in the fibre matrix (Fig. 1 B & C). Water based preparation process with no organic solvents

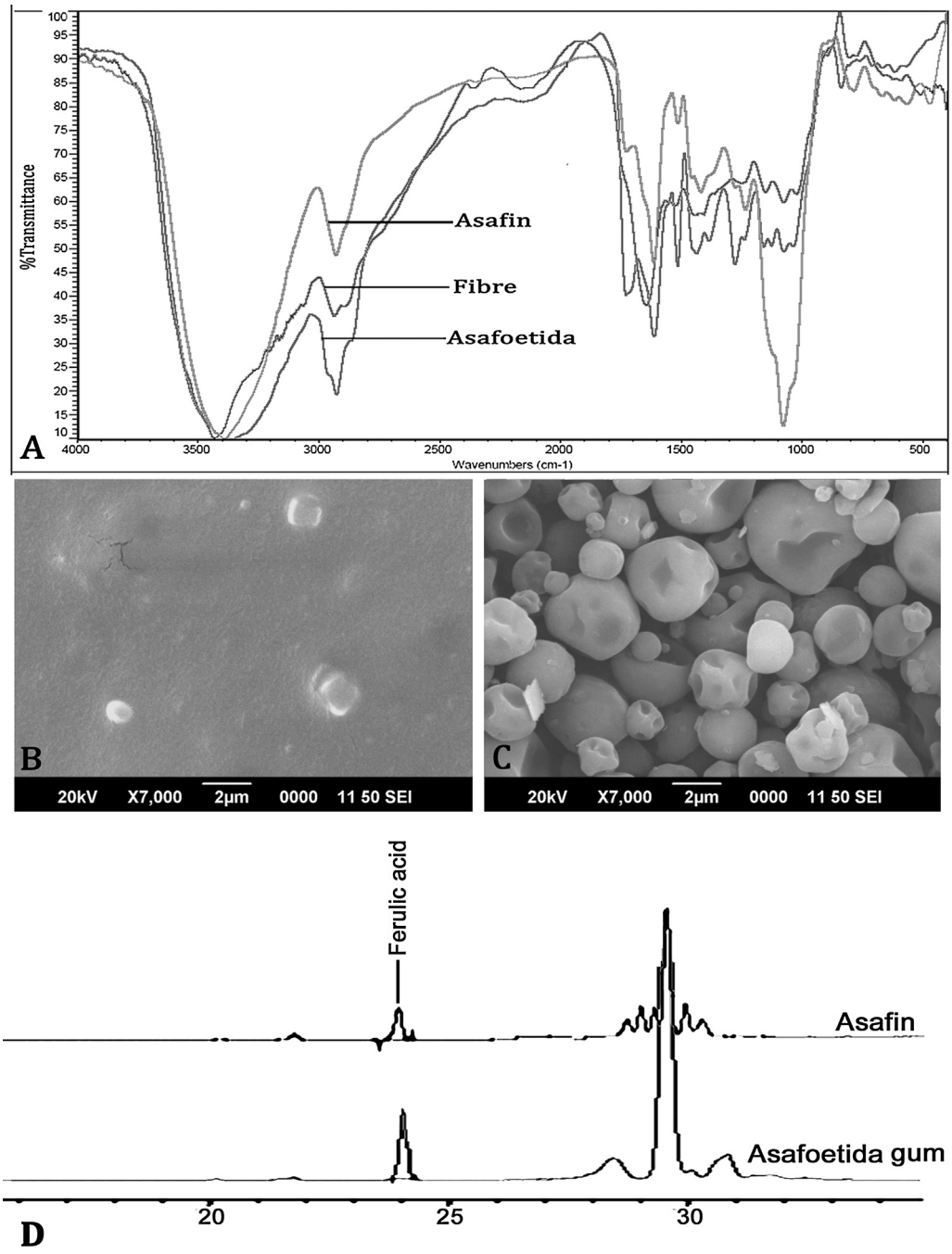

Fig. 1. Characterization of Asafin. **(A)** FTIR spectra of asafetida gum, fenugreek galactomannan fibre and Asafin; **(B)** SEM photograph of Asafoetida gum and **(C)** SEM photograph of Asafin; **(D)** HPLC profile of Asafin and Asafoetida gum.

and synthetic chemicals made the formulation a green process suitable for food and nutraceutical applications. HPLC analysis indicated the stable incorporation of asafetida without degradation (Fig. 1D). Accelerated stability studies for 6 months further demonstrated the stability of Asafin, suitable for a two year shelf-life period, under ambient conditions (Table 1). Asafin was found to have a composition of 58.6% dietary fibre, 4.2% of hexane soluble fraction (fat), 3.5% of volatile oil, 5.8% of proteins with 2.9% of moisture content and 0.61 g mL^{-1} bulk density. Total aerobic plate count, total yeast/mold counts and coliforms were < 350 cfu g^{-1}, < 30 cfu g^{-1} and < 3 MPN g^{-1} with no traces of *E. coli, Salmonella* or the like, indicating the adherence to standard microbial specification for food ingredients (Table 1).

3.1. Toxicity studies

Administration of Asafin at 1 to 4 g Kg^{-1} b.w. was found to induce no mortality, changes in clinical and/or behavior signs or adverse reactions during the lethal dose study. Sub-acute toxicity studies at doses 0.5, 1.0 and 2.0 g kg^{-1} b.w., also did not induce any mortality, abnormal clinical and/or behavioral signs or change in body weight as compared to that of the control group of animals. Body weight and food consumption of the animals also remained unchanged, indicating the safety of Asafin irrespective of the sex of animals.

During the 28-days repeated dose sub-acute toxicity studies, growth rate of normal male rats was found to be 2.35 ± 0.23 g per day and that of female rats were 2.22 ± 0.18 g/day respectively. When treated with Asafin at 2.0 g kg^{-1} b.w. the body weight of male rats were found to be increased from 158.13 ± 9.75 to 223.68 ± 8.95 with a growth rate of 2.34 ± 0.24 per day and that of female rats increased from 154.25 ± 7.74 to 217.63 ± 9.22 with a growth rate of 2.26 ± 0.37 per day, respectively. Either the growth rate or body weight gain remained unchanged in both male and female animals administered with

Table 1
Stability study of Asafin performed as per an in-house protocol developed on the basis of ICH guidelines. The samples were incubated at 40 ± 2 °C and 65% ± 5% RH for a period of 6 months.

Parameter	0 month	1 month	2 months	3 months	6 months
Appearance	Light Brown	Light Brown	Light Brown	Light Brown	Light Brown
Odour	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Moisture (%)	2.90	3.00	3.00	3.10	3.20
Bulk density (g mL^{-1})	0.61	0.61	0.61	0.61	0.61
Protein (g per 100 g)	5.8	5.7	5.7	5.6	5.6
Fibre (%)	58.6	58.6	58.6	58.6	58.6
Fat (%)	4.20	4.20	4.20	4.20	4.20
Volatile oil (%)	3.50	3.48	3.45	3.45	3.40
Microbiology					
Total plate count	< 350 cfu g^{-1}	< 400 cfu g^{-1}	< 350 cfu g^{-1}	< 400 cfu g^{-1}	< 400 cfu g^{-1}
Yeast & Mould	< 30 cfu g^{-1}	< 30 cfu g^{-1}	< 30 cfu g^{-1}	< 30 cfu g^{-1}	< 30 cfu g^{-1}
Coliforms	< 3 MPN g^{-1}	< 3 MPN g^{-1}	< 3 MPN g^{-1}	< 3 MPN g^{-1}	< 3 MPN g^{-1}
E. coli	Absent/g	Absent/g	Absent/g	Absent/g	Absent/g
Salmonella	Absent/g	Absent/g	Absent/g	Absent/g	Absent/g

Table 2
Effect of administration of Asafin (28 days) on haematological and biochemical parameters in Wistar rats (Male and Female).

Parameters	Normal		Asafin 2 g kg^{-1}		Asafin 1 g kg^{-1}		Asafin 0.5 g kg^{-1}	
	Male	Female	Male	Female	Male	Female	Male	Female
Hematology								
Hb (g dL^{-1})	14.24 ± 1.46	13.82 ± 1.10	15.15 ± 0.95	14.35 ± 1.11	15.45 ± 0.97	14.50 ± 1.21	15.1 ± 1.24	14.16 ± 1.20
WBC × 10^3 $(mm^3)^{-1}$	9.12 ± 3.20	8.54 ± 2.61	8.90 ± 3.32	9.08 ± 1.81	9.43 ± 2.44	8.82 ± 2.95	9.20 ± 3.32	8.78 ± 1.80
RBC × 10^6 $(mm^3)^{-1}$	6.86 ± 1.21	7.01 ± 1.80	7.20 ± 1.31	6.79 ± 2.43	6.52 ± 0.95	7.20 ± 2.10	7.18 ± 1.25	6.86 ± 1.22
Platelet × 10^5 $(mm^3)^{-1}$	4.35 ± 1.12	3.81 ± 0.80	4.41 ± 0.79	4.21 ± 1.07	4.90 ± 0.89	5.15 ± 0.95	4.82 ± 0.68	4.16 ± 0.97
Lymphocytes $(mm^3)^{-1}$	6915 ± 1332	6350 ± 1670	7126 ± 1264	6825 ± 1574	6738 ± 1310	6581 ± 1715	7082 ± 1621	6210 ± 1136
Eosinophils $(mm^3)^{-1}$	325 ± 132	415 ± 187	357 ± 126	423 ± 168	366 ± 113	393 ± 120	405 ± 164	384 ± 153
Neutrophils $(mm^3)^{-1}$	1849 ± 520	1632 ± 442	1724 ± 465	1658 ± 535	1782 ± 357	1805 ± 426	1812 ± 512	1671 ± 454
Biochemical								
SGPT (U L^{-1})	34.22 ± 4.51	29.54 ± 3.26	31.30 ± 5.50	31.36 ± 4.29	27.00 ± 4.96	29.38 ± 5.40	32.33 ± 6.56	30.82 ± 3.55
SGOT (U L^{-1})	194.75 ± 26.18	173.20 ± 23.12	185.62 ± 31.41	168.32 ± 34.17	159.50 ± 28.22	171.75 ± 19.85	167.40 ± 26.30	156.20 ± 25.45
ALP (U L^{-1})	295.28 ± 35.19	320.14 ± 49.25	280.18 ± 38.50	293.05 ± 45.14	286.50 ± 24.84	275.20 ± 39.78	298.20 ± 40.53	310.68 ± 51.83
Bilirubin (mg dL^{-1})	0.50 ± 0.04	0.48 ± 0.06	0.40 ± 0.07	0.51 ± 0.05	0.46 ± 0.08	0.38 ± 0.04	0.42 ± 0.05	0.44 ± 0.06
Albumin (g dL^{-1})	4.24 ± 0.87	3.91 ± 0.74	3.55 ± 0.29	4.06 ± 0.58	4.32 ± 0.66	3.89 ± 0.42	3.76 ± 0.97	4.12 ± 0.85
Globulin (g dL^{-1})	2.81 ± 0.48	2.95 ± 0.95	2.59 ± 0.64	3.04 ± 0.81	2.88 ± 0.57	2.45 ± 0.60	2.92 ± 0.78	3.10 ± 0.65
A/G ratio	1.49:1 ± 0.19	1.33:1 ± 0.15	1.37:1 ± 0.22	1.34:1 ± 0.18	1.50:1 ± 0.35	1.59:1 ± 0.32	1.29:1 ± 0.16	1.33:1 ± 0.18
Cholesterol(mg dL^{-1})	68.33 ± 5.45	61.08 ± 7.56	58.74 ± 6.29	60.25 ± 9.43	64.50 ± 8.35	56.25 ± 10.41	67.20 ± 8.92	65.80 ± 11.43
Triglycerides (mg dL^{-1})	62.15 ± 17.28	55.76 ± 14.47	66.50 ± 11.24	46.60 ± 12.43	67.75 ± 14.35	71.25 ± 24.94	56.80 ± 12.91	61.65 ± 13.20
HDL (mg dL^{-1})	32.42 ± 3.27	28.53 ± 5.14	33.20 ± 6.41	36.00 ± 2.71	38.21 ± 4.82	31.06 ± 3.64	39.45 ± 5.14	32.28 ± 4.29
LDL (mg dL^{-1})	46.54 ± 4.39	41.60 ± 6.32	38.33 ± 5.56	40.49 ± 6.25	43.28 ± 7.16	39.75 ± 6.25	44.40 ± 4.39	41.20 ± 7.65
VLDL(mg dL^{-1})	12.32 ± 4.15	10.45 ± 5.29	9.75 ± 3.76	9.24 ± 4.98	11.50 ± 3.18	8.98 ± 5.14	11.43 ± 6.55	10.43 ± 4.17
Urea (mg dL^{-1})	15.48 ± 8.22	18.27 ± 6.48	13.85 ± 7.33	11.21 ± 5.28	14.75 ± 6.24	16.79 ± 6.82	14.64 ± 5.39	12.80 ± 5.46
Creatinine (mg dL^{-1})	0.58 ± 0.09	0.52 ± 0.06	0.54 ± 0.05	0.50 ± 0.05	0.48 ± 0.06	0.53 ± 0.07	0.46 ± 0.09	0.49 ± 0.08
Na^+ (m mol L^{-1})	138.00 ± 5.28	143.20 ± 8.30	135.67 ± 6.25	139.25 ± 8.15	141.19 ± 7.56	137.25 ± 6.09	142.15 ± 5.98	140.24 ± 6.80
K^+ (m mol L^{-1})	5.08 ± 0.14	4.32 ± 0.22	5.13 ± 0.27	4.12 ± 0.56	4.05 ± 0.75	5.10 ± 0.63	4.35 ± 0.40	3.94 ± 0.84
Cl^+ (m mol L^{-1})	99.57 ± 6.92	102.10 ± 5.60	98.77 ± 7.58	100.49 ± 6.24	103.38 ± 7.42	98.03 ± 8.05	97.43 ± 6.71	101.08 ± 4.97
HCO_3^+ (m mol L^{-1})	23.17 ± 4.23	24.00 ± 2.91	25.05 ± 3.13	22.45 ± 4.10	25.18 ± 3.48	23.68 ± 2.93	24.25 ± 4.29	22.86 ± 1.98

The values are expressed as mean ± SD for 5 animals in each group.

Table 3
Ulcer index and percentage inhibition of alcohol-induced ulcer upon Asafin-treatment.

Treatment	Dose (mg kg^{-1} b.w.)	Ulcer index	Percentage Inhibition (%)
Pre-treatment	Ethanol	4.47 ± 0.37	–
	Ranitidine (50)	1.9 ± 0.51	56.8
	Asafin (50)	4.0 ± 0.29	9.0
	Asafin (100)	2.6 ± 0.68	40.9
	Asafin (250)	4.0 ± 0.41	85.2
Post-treatment	Ethanol	4.47 ± 0.37	–
	Ranitidine (50)	1.81 ± 0.39	52.4
	Asafin (50)	3.37 ± 0.25	14
	Asafin (100)	1.73 ± 0.37	55
	Asafin (250)	0.26 ± 0.18	94.5

Asafin (0.5, 1.0 and 2.0 g kg^{-1} b.w.) when compared to the normal. Haematological and biochemical parameters of Asafin treated rats were also remained in the normal range without significant ($p > 0.05$) changes (Table 2). Biochemical parameters related to hepatic and renal function were in the normal range. Renal profile, liver function markers, creatinine, urea or the serum electrolyte levels in both male and female rats were comparable to the normal animals even after 28 days of Asafin supplementation. Similarly the lipid profile which also remained unchanged with no significant ($p > 0.05$) variation in cholesterol, HDL, LDL and VLDL levels in both male and female rats when treated with Asafin (Table 2). Necropsy of the treated animals showed normal appearance for various organs and tissues (Table 3). The weight (g) of the vital organs comprising liver, spleen, kidney and brain of normal male rats were 3.21 ± 0.36, 0.32 ± 0.05, 0.84 ± 0.09 and

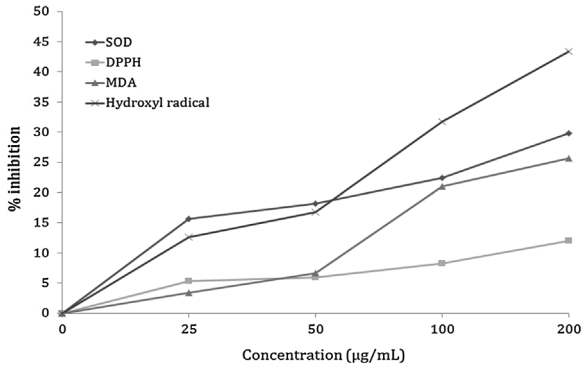

Fig. 2. *In vitro* antioxidant efficacy of Asafin.

0.68 ± 0.17 and that of female rats were 3.16 ± 0.24, 0.29 ± 0.05, 0.76 ± 0.27 and 0.72 ± 0.14 respectively. Upon Asafin treatment at 2.0 g kg^{-1} b.w., the organ weights of male (3.26 ± 0.41, 0.34 ± 0.08, 0.78 ± 0.23 and 0.71 ± 0.12) and female (3.13 ± 0.21, 0.33 ± 0.06, 0.74 ± 0.18 and 0.65 ± 0.13) rats did not show any significant changes as compared to normal rats.

3.2. *In vitro* antioxidant activities

Asafin showed significant radical scavenging activities and anti-oxidant efficacy against various *in vitro* assays, as evident from Fig. 2. IC_{50} values for the superoxide radical scavenging ability, inhibition of hydroxyl radicals, and DPPH radical scavenging activities were found to be around 290 µg mL^{-1}, 213 µg mL^{-1}, and 570 µg mL^{-1} respectively. However, the IC_{50} values for the water soluble vitamin C, under same experimental conditions, were 780 µg mL^{-1}, 965 µg mL^{-1}, and 38 µg mL^{-1} respectively. Further investigations on the effect of Asafin on the inhibition of lipid peroxidation in comparison with vitamin C, using incubation of rat liver homogenates with the oxidant chemical species Fe^{2+}, showed IC_{50} values of 375 µg mL^{-1} and 350 µg mL^{-1} respectively for vitamin C and Asafin (Fig. 2).

3.3. *Anti-inflammatory activity*

Anti-inflammatory effects followed by the oral administration of Asafin at 250 and 500 mg kg^{-1} b.w. were evaluated by an acute model of carrageenan-induced paw edema and formalin induced chronic model of mice.

3.3.1. *Carrageenan induced acute inflammation model*

The sub-plantar injection of carrageenan induced severe edema in mice paws with a maximum at 3 h post-injection. In the present study, the relative inhibition of edema by the oral administration of Asafin (200 and 500 mg/kg b.w) and *i. p.* administration of diclofenac at 10 mg kg^{-1} b.w. were compared at different time intervals for up to 6 h and 24 h of post-dose (Fig. 3A). It was observed that Asafin provided a significant ($p < 0.05$) inhibition of 21.5 and 28.9% ($p < 0.05$) respectively, when compared to the control group after 6 h of edema induction. The standard drug diclofenac given as *i.p.* injection resulted a 23.4% inhibition in edema.

3.3.2. *Formalin induced chronic inflammation model*

Asafin showed a significant ($p < 0.01$) suppression of formalin induced inflammation in a concentration dependent manner. Relative percentage inhibitions observed on the 7th day of formalin injection were 38 and 49.2% respectively for doses 250 and 500 mg kg^{-1} b.w (Fig. 3 B). The inhibition for standard drug diclofenac (10 mg kg^{-1}) was 28%.

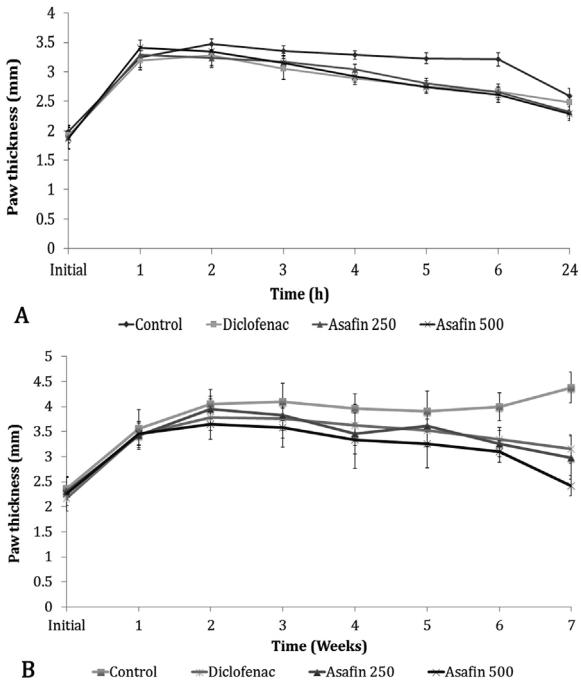

Fig. 3. Effect of Asafin on **(A)** carrageenan-induced and **(B)** Formalin-induced paw edema in Swiss albino mice. The values are expressed as mean ± SD for six animals in each group.

3.4. *Anti-nociceptive activity*

A significant ($p < 0.01$) reduction in the number of writhing moves induced by acetic acid was observed when Asafin was orally administered. The relative percentage reduction was 38.5 and 55.6% respectively for doses of 250 and 500 mg kg^{-1} b.w. However, the reduction in writhing observed for the control drug aspirin at 10 mg kg^{-1} *i.p.* administration was 63.3%.

3.5. *Anti-ulcer activity*

3.5.1. *Anti-ulcerogenic effect followed by 'Asafin' pre-treatment (Preventive effect)*

Pretreatment of Asafin was found to have a significant effect upon ethanol-induced gastric ulcer formation. While the normal control group of animals produced severe necrotic lesions throughout the glandular portion of rat stomach with an ulcer index of 4.4 ± 0.37, ulcer indices upon 50, 100 and 250 mg kg^{-1} b.w. of Asafin were 4.0 ± 0.29 ($p < 0.01$), 2.6 ± 0.68 ($p < 0.01$) and 0.65 ± 0.41 ($p < 0.01$) respectively. The percentage inhibition of ethanol-induced gastric ulcer formation of the corresponding dose was 9.1, 40.9 and 85.2% respectively. The relative ulcer inhibition by the standard drug 'ranitidine' at 50 mg kg^{-1} b.w., was 56.8% with an ulcer index of 1.9 ± 0.51 ($p < 0.01$). The morphology of the stomach of normal animals, alcohol-induced ulcer bearing animals and Asafin treated animals is depicted in Fig. 4A. Histopathological analysis (Fig. 5) of stomach tissues demonstrated hemorrhagic erosion, discontinuity in the lining of epithelium cells and significant damage in sub and muscularis mucosa among the alcohol treated animals as compared to the normal group. Ranitidine treatment could induce significant protection to the stomach, though mucosal disruption with small atrophic glands and mild hyperplasia. The observation was almost similar when Asafin was treated at 50 mg kg^{-1} b.w. Further improvement was observed upon 100 and 250 mg kg^{-1} b.w. of Asafin treatment with a significant protection in a concentration dependent manner. At 250 mg kg^{-1} b.w., Asafin reduced the gastric lesion formation and sub-mucosal edema better than the standard drug ranitidine (Fig. 4B). Asafin treatment also

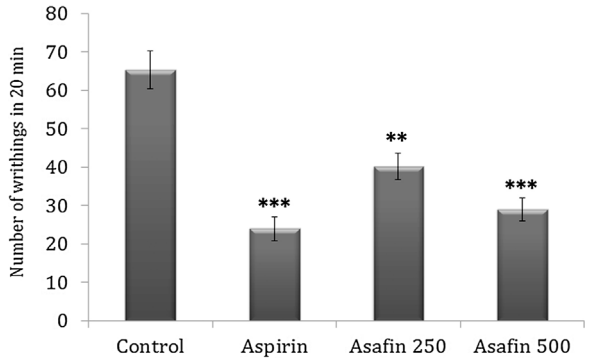

Fig. 4. Analgesic effect of Asafin on acetic acid induced pain in Swiss albino mice. The values are expressed as mean ± SD for six animals in each group, where ** $p < 0.01$ and *** $p < 0.001$, when treated group compared with that of control.

produced a significant dose dependent enhancement in the mucosal volume as compared to ethanol treated group which showed significant decrease as compared to the normal group of animals. The relative mucosal content were 248 ± 15.58 µg Alcian blue g^{-1} wet tissue (normal), 147.5 ± 14.43 µg Alcian blue g^{-1} wet tissue (ethanol control). The pre-administration of Asafin at 100 and 250 mg kg^{-1} b.w. significantly ($p < 0.01$) improved the mucosal volume from 147.5 ± 14.43 µg Alcian blue g^{-1} wet tissue (control) to 225.67 ± 18.25 µg Alcian blue g^{-1} wet tissue and 233.17 ± 11.37 µg Alcian blue g^{-1} wet tissue respectively.

3.5.2. Antiulcer activity of 'Asafin' when administered after ethanol consumption (Curing effect)

Asafin treatment even after 1 h of ethanol administration was also found to provide a significant stomach protection. Ethanol treated group showed severe necrotic lesions throughout the glandular portion of the stomach with an ulcer index of 4.4 ± 0.37. At 250 mg kg^{-1} b.w., Asafin produced an ulcer index of only 0.26 ± 0.18 with 94.1% inhibition. The lower doses 50 and 100 mg kg^{-1} b.w., showed 14 and 55% inhibition respectively against the ranitidine treated group which showed 61% inhibition. Detailed investigation on the mucosal content of the ethanol-administered control group animals and Asafin treated animals also showed a significant dose dependent ($p < 0.01$) enhancement in mucosal content; 228.5 ± 11.49 and 241.33 ± 10.23 µg Alcian blue g^{-1} wet tissue respectively for 100 and 250 mg kg^{-1} b.w. of Asafin.

4. Discussion

The name 'Assa-foetida' is reported to be derived from the Latin word meaning the "carrier of bad smell." It is also true that asafoetida is very often referred to as 'Devil's dunk', indicating the degree of unpleasant organoleptic character it possess. However, it is interesting to note the other common name of asafoetida as 'Food of God', mainly due to the health beneficial effects and medicinal properties. Asafoietida gum has a general composition of 40–65% (w/w) of resin fraction, 25–30% (w/w) of carbohydrates and 10–17% (w/w) of essential oil fraction [12]. The resin fraction contains ferulic acid and its esters, coumarins, sesquiterpenes, and terpenoids [16]. Glucose, galactose, arabinose, rhamnose, glucuronic acids, and their polysaccharides were identified as the major carbohydrates. Sulfur-containing compounds along with terpenes and terpenoids were characterized in the volatile oil fraction and were found to be responsible for the unpleasant flavor characteristics [36]. Asafin was formulated as a free flowing microencapsulated granules which are water dispersible and compressible suitable for capsulation and tableting. Uniform impregnation of the lipophilic gum into the hydrophilic matrix of dietary fibre gel, gave taste and odour masked Asafin particles for oral delivery. Gum like-character, high viscosity, encapsulating effect, mucoadhesive and gastroretentive properties of fenugreek galactomannans have already been

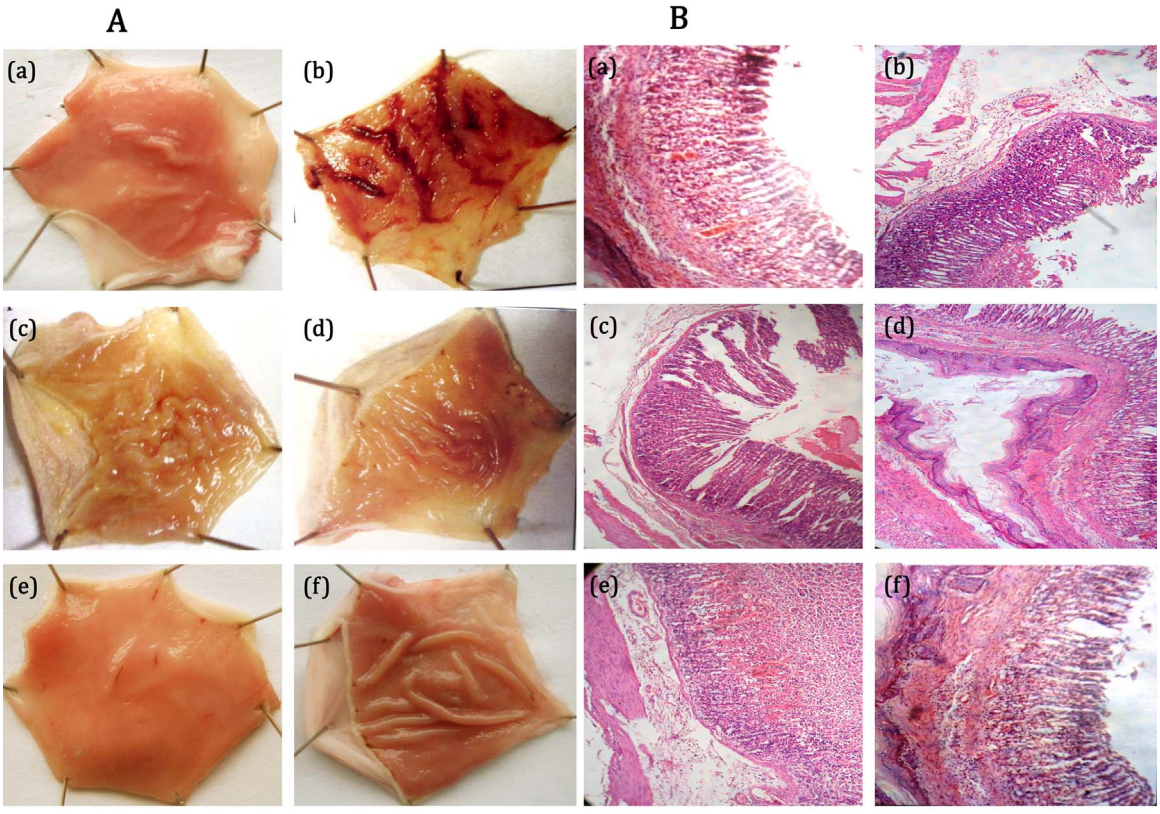

Fig. 5. (A) Morphology and **(B)** histopathology of stomach tissues of Wistar rats treated with (a) normal, (b) ethanol treated control, (c) ranitidine, (d) Asafin – 50 mg kg^{-1} b.w. (e) Asafin – 100 mg kg^{-1} b.w. (f) Asafin – 250 mg kg^{-1} b.w.

reported as a good natural polymer suitable for oral delivery formulations [15]. Relatively high fibre content and also water based process without using any synthetic excipients or organic solvents make it suitable for nutraceutical and food applications. Accelerated stability studies have demonstrated its suitability for long term storage as well.

Despite the various health beneficial pharmacological effects, no stable formulations of asafoetida suitable for human supplementation in various convenient dosage forms have been reported so far. Asafin is a 100% natural food-grade formulation of asafoetida gum. It showed significant *in vitro* antioxidant efficacy and radical scavenging ability comparable to Vitamin C. Asafin also showed significant *in vivo* anti-inflammatory effect in both acute (carrageenan-induced) and chronic (formalin-induced) inflammatory models. While carrageenan-induced paw edema model is a widely used primary test model to screen new anti-inflammatory agents, formalin-induced model was reported to have a close resemblance with human arthritis [37,38]. While carrageenan-induced inflammation is a biphasic process with an early phase (1–2 h) mediated by histamine and prostaglandins, followed by a later phase involving prostaglandins, bradykinin and leukotrienes [37,39], formalin-induced inflammation comprises a neurogenic response mediated by substance P and bradykinin followed by a tissue mediated response where histamine, 5-hydroxytryptamine, prostaglandins and bradykinin are involved [38]. Oral supplementation of Asafin at 250 mg kg^{-1} b.w. could produce an anti-inflammatory response similar to 10 mg kg^{-1} b.w. intraperitoneal administration of diclofenac. But, administration at 500 mg kg^{-1} b.w. did not show a dose response. In formalin–induced model, Asafin showed better reduction in the paw inflammation as compared to the reduction in edema by the standard drug diclofenac at an *i.p.* dose of 10 mg kg^{-1} b.w. Diclofenac is non-steroidal anti-inflammatory drug very often considered as the first choice in the treatment of acute and chronic painful inflammatory conditions [40].

Acetic acid-induced writhing model of mice is regarded as a well validated animal model for peripheral inflammatory pain evaluation of analgesics or anti-inflammatory drugs [41]. The peripheral analgesic is due to the liberation of several inflammatory mediators such as bradykinin, substance-P, prostaglandins, cyclooxygenases, lipoxygenases and some cytokines such as IL-1β, TNF-α and IL-8 [42,43]. Acetic acid was shown to indirectly stimulate the nociceptive neurons by the release of endogenous mediators [44]. The present study has demonstrated significant antinociceptive efficacy of Asafin, possibly due to the inhibition of synthesis of arachidonic acid metabolite mediated by COX inhibition.

One of the wide-spread traditional use of asafoetida gum include its potentiality against digestive disorders [12]. *In vivo* studies have demonstrated the digestive, antispasmodic, carminative and laxative properties of asafoetida gum-resin [12]. Thus, the present study evaluated the gastroprotective activity of Asafin against ethanol-induced stomach ulcers. Heavy consumption of alcohol in empty stomach and alcoholics were shown to have a significant degree of gastric ulcers and inflammation leading to discomforts such as bloating, fullness, gastritis, reduced appetite, and abdominal pain. Ethanol-induced damage was found to be initiated by microvascular injury; namely the disruption of the vascular endothelium resulting in increased vascular permeability, linear hemorrhagic lesions, sub-mucosal edema, mucosal friability and epithelial lifting [1,45,46]. Ethanol produces necrotic lesions in the gastric mucosa reducing the secretion of bicarbonates and production of mucus [47]. Ethanol acts directly on the gastric epithelium and perturb the mast cells leading to the release of a vasoactive mediator such as histamine. Administration of Asafin (250 mg kg^{-1} b.w.) was found to inhibit the ulcer formation almost completely (94%). Asafin also showed anti-ulcer efficacy even at a low dose of 100 mg kg^{-1} b.w. with a significant reduction of ethanol-induced hemorrhage, necrosis and ulceration of the gastric mucosa indicating its potent anti-ulcerogenic efficacy.

5. Conclusions

Botanical extracts derived from food components having a history of safe human consumption are always of great significance as a nutraceutical or functional food ingredient. While consider the increase in alcoholism and its gastrointestinal complications, the results of the present study indicates the gut health potential of Asafin; a 100% natural green formulation employing the fenugreek derived soluble dietary fibre (galactomannans), with enhanced stability, water solubility and minimized organoleptic properties. The significant ability of Asafin to reduce the ulcer areas in the gastric wall as well as to inhibit the edema and leucocytes infiltration in the submucosal layers in a dose dependent manner could be of great interest as a nutraceutical and/or functional food ingredient to alleviate gut disorders originating from diet, alcoholism, smoking and chemical drugs.

Conflict of interest

The authors declared the potential conflict of interest. Asafin™ is the registered trademark name of M/S. Akay Flavours & Aromatics Ltd, Cochin, India. VBL, IJJ and RK belongs to a non-profitable research organization and have no conflicts of interest.

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