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EFFECTS OF *PORPHYRA VIETNAMENSIS* EXTRACT ON TNBS-INDUCED COLITIS IN RATS

S. Bhatia [*1], S. Sardana [1], T. Naved [2] and A. Sharma [3]

Amity Institute of Pharmacy [1], Amity University, Manesar - 122413, Haryana, India.
Amity Institute of Pharmacy [2], Amity University, Noida - 201313, Uttar Pradesh, India.
Amity Institute of Pharmacy [3], Amity University, Gwalior - 474020, Madhya Pradesh, India.

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Correspondence to Author:
Dr. Saurabh Bhatia

Associate Professor,
Amity Institute of Pharmacy,
Amity University, Manesar - 122413,
Haryana, India.

E-mail: sbsaurabhbhatia@gmail.com

ABSTRACT: Objective: This study aimed to investigate the intestinal anti-inflammatory activity of a methanolic extract of *Porphyra vietnamensis* in the TNBS model of intestinal inflammation in rats. **Methods:** The study included (i) Identification and quantification of Porphyra-334 in *P. vietnamensis*, (ii) investigation of the effect of methanolic extract on DNA cleavage induced by H_2O_2 UV-photolysis, (iii) 500 µg/ml *Porphyra* methanolic extract (PE) loaded 20% pluronic F127 gel was prepared, and its gelation, gel melting temperatures as well as bio-adhesive strength were determined, (v) anti-inflammatory potential of PE against the intestinal inflammatory process induced by TNBS (trinitro-benzesulphonic acid) in rats was evaluated. The protective effects were evaluated as follows: evaluation of intestinal damage (damage score, colon weight) and adherence to adjacent organs, colon malondialdehyde (MDA) estimation. **Results:** 0.0317% w/w of P-334 with R_f value of 0.48 was calculated from the methanolic extract of *Porphyra.* PE showed a dose-dependent (300-500 µg/L) protective effect on DNA cleavage. In contrast with Mesalamine (a standard anti-inflammatory drug), treatment with purified P-334 and gel (500 µg/L) formulation containing PE showed significant protective effects in the TNBS-induced colon damage. PE treatment positively scored on histopathological parameters. The post-treatment intestinal features showed restoration at par with the healthy intestine. **Conclusion:** 1000 mg of PE per kg rat weight showed significant mucorestorative in TNBS-induced colonic damage rats. The observed anti-inflammatory and antioxidant effects may be associated with the presence of P-334 in *P. vietnamensis*.

INTRODUCTION: Inflammatory bowel disease (IBD) is a widely chronic and multifactorial gastrointestinal (GI) inflammatory condition which is categorized into ulcerative colitis (UC) and Crohn's disease (CD) in the clinic. Etiology and pathophysiology of IBD are still unknown and multifactorial [1].

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This condition is driven by intestinal immunity, inflammatory mediators, oxidative stress, colonic milieu, the content of the mucosa, intestinal permeability, sulfide production, and decreased methylation [2].

Current treatments include commonly used drugs, such as aminosalicylates, which assist in maintaining remission of crises, corticosteroids, which are utilized during acute episodes, and immunomodulators [3]. However, these treatments are often associated with severe side effects and high costs [4, 5]. Thus, there is a search for safe, natural compounds that can contribute to the

prevention or even treatment of inflammatory diseases [6]. Mycosporine-like amino acids (MAAs) are a family of intracellular compounds biosynthesized by shikimic acid pathway for the synthesis of aromatic amino acids involved in the protection of aquatic organisms against solar radiation. They are small (<400 Da), colorless and highly polar substances having absorption maxima ranging from 310 to 360 nm, with an average molecular weight of around 300 Da. Till now, the chemical structures of over 30 different mycosporines have been elucidated, and almost all MAAs follow a common isolation procedure [7].

They have been identified in some taxonomically diverse organisms such as fungi, marine heterotrophic bacteria, cyanobacteria, eukaryotic marine invertebrates and a wide variety of other marine organisms. Their highly photoprotective favorable properties such as high molar coefficients (ε=28,100–50,000/M/cm), strong UV-absorption maxima and photostability, and resistance to abiotic stressors increase their demand as effective sunscreening compounds [7]. Their antioxidant nature increases the therapeutic effectiveness [8]. Recently introduced porphyra-334 **Fig. 1** isolate from Indian species of *Porphyra vietnamensis* has given excellent sun-protective effects against widely used *Aloe vera* gel.

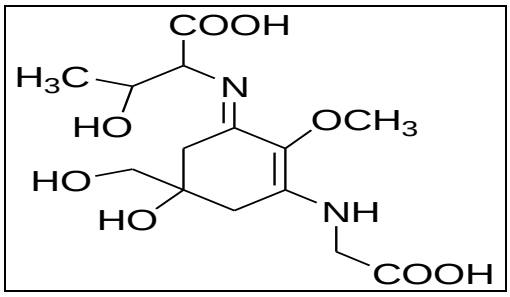

FIG. 1: PORPHYRA-334 (MYCOSPORINE LIKE COMPOUNDS)

Our previous and other studies proved that Porphyra-334 (P-334) which is widely present in the nutritious and therapeutic Porphyra [9-12, 21-33] is having high antioxidant [8, 21-33] and sun-protective potential [9, 21-33]. These properties make it suitable for the evaluation of its effectiveness against TNBS induced colitis in rats.

MATERIALS AND METHODS:

Extraction Procedure: *P. vietnamensis* was collected from different locations of Ratnagiri, Maharashtra and authentified. 19 mg of dried and purified compound was isolated from 1.7 gram of lyophilized pale yellow powder (which was obtained from 5 gram of dried algal powder) according to the procedure adopted in our previous studies [9].

HPTLC Analysis: The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60 $_{F-254}$ plates, [10 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany)] using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110 ºC for 5 min before chromatography. A constant application rate of 0.1 µL.s^{-1} was used, and the space between two bands was 5 mm. The slit dimension was kept at 5 mm × 0.45 mm and the scanning speed was 10 mm.s^{-1}.

The monochromator bandwidth was set at 20 nm, each track was scanned three times, and a baseline correction was used. The mobile phase consisted of MeOH: acetonitrile: water (50:40:10) and chromatograms were monitored at 334 nm [9], 10 mL of mobile phase was used per chromatography run. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25 ºC ± 2) at a relative humidity of 60 % ± 5. The length of each chromatogram run was 8 cm. Following the development, the HPTLC plates were dried in a current of air with the help of an air dryer in a wooden chamber with adequate ventilation.

The flow rate in the laboratory was maintained unidirectional (laminar flow, towards the exhaust). Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 334 nm and operated by CATS software (V 3.15, Camag). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the compound chromatographed were determined from the intensity of the diffused light. The evaluation was by peak areas with linear regression.

Calibration Curve: 100 ug/mL of a stock solution containing P-334 reference standard (isolated and purified in our lab), was prepared in methanol and aliquots of 2-14 µL were applied to the TLC plate to get a final concentration of 200-1400 ng/spot. The plates were developed as per the conditions mentioned above. The graph was plotted for peak area versus concentration, and the data was treated by linear regression analysis. The regression equation obtained was further used for the quantitative determination of P-334 from the metabolic extract of *P. vietnamensis*.

DNA Cleavage Assay (Antioxidant Assay): The UV irradiation of DNA in the presence of H_2O_2 resulted in the cleavage of supercoiled DNA (scDNA) to open chain DNA (ocDNA) and linear DNA (lin form), indicating that OH^- generated from UV photolysis of H_2O_2 produced DNA strand scission or cleavage [13]. In this assay 25 mg of methanolic extract, the powder was dissolved in 10 ml of ethanol and various concentrations of extracts such as 100, 250, 500, 1000, 1500, 2000, 2500 µg/ml solutions were prepared. The experiment was performed in a volume of 20 µl containing 400 ng/µl of pUC 18 plasmid DNA in 10 mM Tris HCl & 1mM EDTA, pH 7.6, in the presence of different concentration (100-500 µg/ml) of PE. Immediately before irradiating the samples with UV light, H_2O_2 was added to final concentration of 2.5 mM. The reaction volumes were held in caps of polyethylene microcentrifuge tubes, that were placed directly on the surface of a transilluminator (8000 u w cm^{-1}) at 600 nm. The samples were irradiated for 2 min at room temperature.

After irradiation 5 µl of a mixture, containing 0.25% bromophenol blue and 60% glycerol were added to the irradiated solution. The samples were then analyzed by electrophoresis on a 1% agarose horizontal slab gel in Tris-borate buffer (45 mM Tris-borate, 1 mM EDTA). Untreated pUC 18 plasmid was included as a control in each run of gel electrophoresis, which was carried out at 50 V/cm for 6 h. A gel was stained in ethidium bromide.

TNBS-Induced Experimental Colitis Model: To study the use of 5-ASA for targeted drug delivery to the inflamed tissue of colon in IBD, trinitrobenzene sulfonic acid (TNBS) induced experimental colitis model was selected because site specificity can only be studied by treating the inflammation that occurs in the colon. This new model is simple and reproducible. Moreover, it is the most relevant model as it involves the use of immunological haptens and develops a chronic inflammation rather than an acute mucosal injury [14]. This model would allow an *in-vivo* characterization of the azo carrier system under the influence of chronic inflammatory symptoms. To explore the pathogenesis of inflammatory bowel disease (IBD) and to establish some indices monitoring the activity and severity of IBD, TNBS induced colitis model was established. In this, enteritis based on a delayed-type of hypersensitivity reaction was induced by TNBS (2, 4, 6-trinitrobenzene sulfonic acid). This treatment induced a chronic inflammation of the colon, characterized by gross hyperemia and edema, as assessed by a macroscopic score. Histologically, the inflammatory response included cell infiltration by lymphocytes and histiocytes, a transmural granulomatous inflammation with multinucleated cells and activated mesenteric lymph nodes.

Induction of Colitis: The experimental animal procedures were by the regulation of institutional animal ethical committee, PDM College of Pharmacy, (PDM/CPCSEA/RES/ 2013/01). Male Wistar rats (average weight 200–230 g; 12-15 weeks; n=6/group) were used. They were distributed into 4 different groups,' *i.e.,* healthy control, colitis control, positive control, and test group. They were housed in a room with controlled temperature. The animals were food fasted 48 h before experimentation and allowed food and water after the administration of TNBS.

To induce an inflammation, all the groups except healthy control group were treated by the following procedure: after light narcotizing with ether, the rats were catheterized 8 cm intrarectal and 0.25 ml of TNBS in ethanol was injected into colon *via* rubber cannula (dose was 100 mg/kg of body weight of TNBS in 50% v/v ethanol solution). Animals were then maintained in a vertical position for 30 sec and returned to their cages. For 3 days the rats were housed without treatment to maintain the development of a full inflammatory bowel disease model.

The animals of positive and test groups were received Mesalamine enema (Mesacal R), Purified

Porphyra-334 (P-334) and test formulation rectally, respectively, once. No drug treatment was given to healthy control and colitis control.

Preparation of P-334 Gel for Rectal Application: Gels were prepared on weight basis using the cold method. Amount of Pluronic F127 sufficient to yield desired [20 (% w/w)] gel was slowly added to cold water (5 ºC) maintaining constant stirring. Each dispersion was refrigerated until a clear solution was formed (5 h). The concentration of methanolic extract of *Porphyra* (1 gm/kg body weight) was kept constant in all the batches.

Evaluation Gelation and Gel Melting Temperature: Gelation and gel melting were assessed using a modification of Miller and Donavan technique. 5 ml aliquot of the gel was transferred to test tubes, immersed in a water bath at 4 ºC and sealed with aluminum foil. The temperature of the water bath was increased in increments of 1 ºC and left to equilibrate for 1 min at each new setting. The samples were then examined for gelation, which was said to have occurred when the meniscus would no longer move upon tilting through 90º. The gel melting temperature, a critical temperature when a gel starts flowing upon tilting through 90º was recorded.

Evaluation of Bio-Adhesive Strength of Prepared Formulation: The bio-adhesive strengths of prepared gels were estimated regarding maximum adhesion force and work of adhesion shear required to separate the gel from sheep intestinal mucosa. The method developed by Jimenez-Castellanos and co-workers was modified and used (Chi-Hyun Lee, 1996). The bioadhesive strength was investigated by using force gauge instrument (Advanced Force Gauge, Mecmesin, West Sussex, England) equipped with a 50 N load cell and metal probe (diameter, 5 mm; length, 15 cm). In this study, two pieces of the wooden block were bound to the external surface of each piece of colonic mucosa (10 cm × 2.5 cm).

The lower wooden block was fixed to the flat surface, and the upper block was fixed to the metal probe. The formulation was applied to the internal mucosal surface of the lower wooden block. Then these two pieces of wooden blocks were maintained in contact, a constant weight (625 gm) was kept on the upper wooden block for a constant period (10 min). The probe was then driven in an upward direction at a constant rate of 200 mm/min. In this test, we have optimized the time for which two mucosal surfaces were in contact with each other and rate with which probe was driven in an upward direction. Stress was thus imposed by linear displacement of the sample until fracture of the adhesive bond occurred, and the adhesion force *vs*. time profile was monitored.

Assessment of Colonic Damage by Clinical Activity Score: The animals of all groups were examined for weight loss, stool consistency, and rectal bleeding throughout the 11 days study. At the end of the experiments, rats were sacrificed by cervical dislocation, the colon excised, opened longitudinally, and washed in saline. Macroscopic damage was assessed on the basis of a semi-quantitative scoring system, [16] which takes into account the area of inflammation and presence/absence of ulcers as described by Ukil *et al*., [17] (No ulcer, no inflammation: 0; No ulcer, local hyperemia: 1; Ulceration without hyperemia: 2; Ulceration and inflammation at one site only: 3; Ulceration and inflammation at two or more sites: 4; and Ulceration extending more than 2 cm: 5). A 10 cm segment of the colon was excised and weighed as an increase in weight is seen after the induction of colitis.

Further, these weights were compared with different groups to give an idea about weight recovery and malondialdehyde levels determined in each tissue, which served as an indicator of lipid peroxidation.

Histopathological Analysis: Animals were sacrificed within 24 h after the last drug administration on last day, and a segment of colon 8 cm long was excised Tissue segments 1 cm in length was then fixed in 10% buffered formalin for histopathological studies. The histopathological sections were stained with hematoxylin and eosin, and colored microscopical images of the colon sections were taken on the optical microscope with resolution 10X - 45X fitted with a trinocular camera.

Statistical Analysis: All antioxidant assays were carried out in triplicate (n=3). Results are expressed

as mean ± standard deviation of n observations. We used analysis of variance to determine the statistical significance of intergroup comparisons. $P<0.05$ was considered to be statistically significant. Macroscopic and microscopic scores for colonic erosions for the *P. vietnamensis* - pretreated groups were compared against those for the TNBS-treated group with a one way ANOVA Tukey test. All the statistical analysis was carried out using graph pad (Instat software, USA) version 3.

RESULTS AND DISCUSSION:

HPTLC Analysis: In the present study, MAA, known to be present in macroalgae of *P. vietnamensis*, was detected and quantified by HPTLC (R_f-0.48) fingerprinting. To investigate the presence of various chemical constituents in *Porphyra* extract HPTLC fingerprinting was carried out. An HPTLC method was developed for quantification of P-334 from the alcoholic extract of P-334 using methanol: acetonitrile: water (50:40:10) as a solvent system and R_f value of P-334 is 0.48.

The representative densitogram and a calibration curve of standard P-334 shown in supplementary file (data shown in supplementary file).

Quantitative investigation for P-334 content in methanolic extract of *P. vietnamensis* was carried out using the HPTLC method described above. The peak at R_f 0.48 in the methanolic extract was considered as P-334 which was further confirmed by the overlay spectra of standard and sample **Fig. 3** (data are shown in supplementary file). The percentage with P-334 content was calculated using the regression equation and was found to be 0.0317% w/w. The representative densitogram and the overlay spectra of PE are shown in the supplementary file.

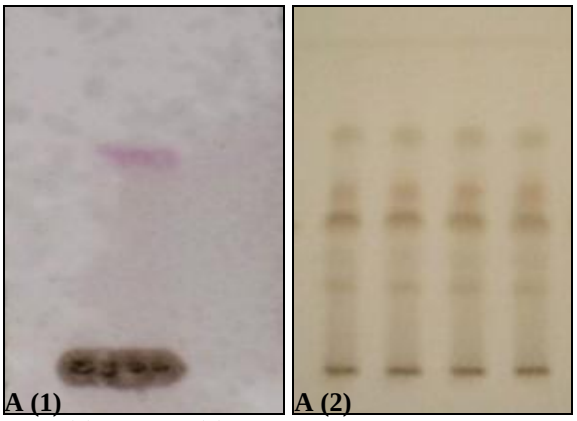

FIG. A (1) & A (2): HPTLC OF PORPHYRA-334 STANDARD COMPOUND AND METHANOLIC EXTRACT OF *P. VIETNAMENSIS.*

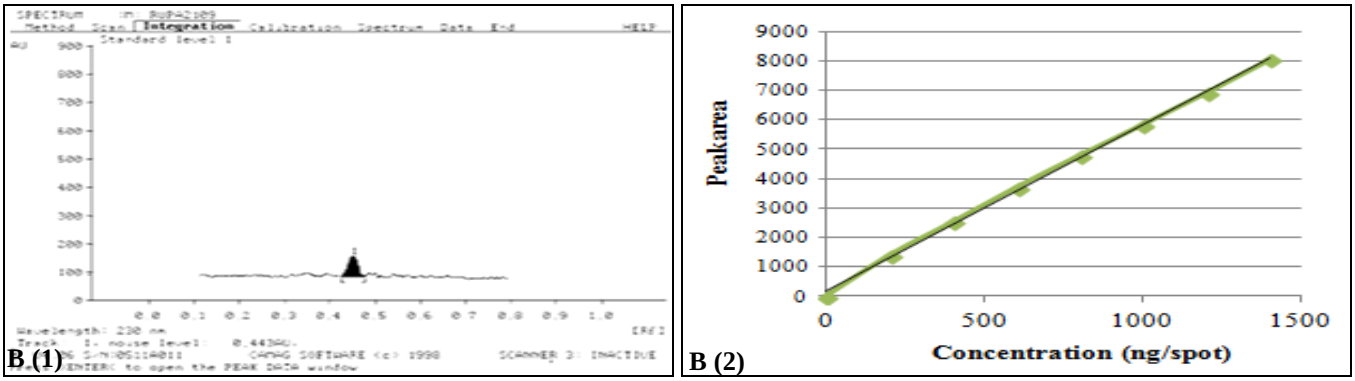

FIG. B(1) & B(2): DENSITOGRAM AND CALIBRATION & CURVE FOR PORPHYRA-334

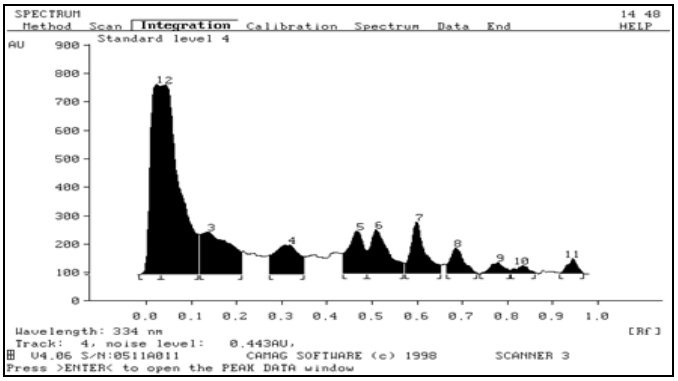

FIG. C. DENSITOGRAM OF METHANOLIC EXTRACT OF *PORPHYRA* EXTRACT

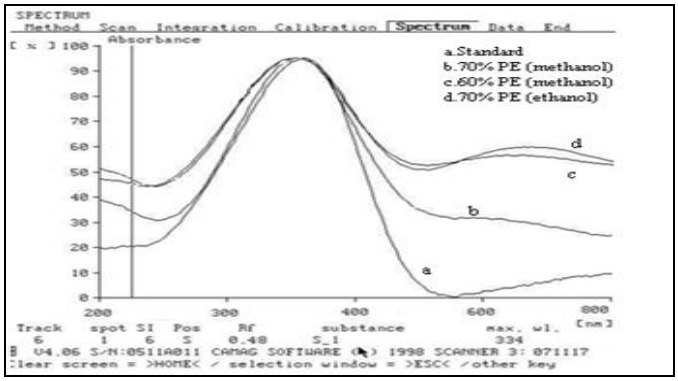

FIG. D. SPECTRA OVERLAY OF STANDARD AND DIFFERENT ALCOHOLIC EXTRACTS FROM *P. VIETNAMENSIS* AT 334 nm

DNA Cleavage Assay: The electrophoretic pattern obtained after UV-photolysis and radical hydrolysis of DNA [H_2O_2 (2.5mM)] in the absence and presence of PE (300-500 µg/ml) is shown in **Fig. 2**. Intact band of DNA derived from pUC 18 plasmid was initially obtained (lane a). The faster-moving band corresponds to the native form of super coiled circular DNA (sc DNA), and the slower moving band was the open circular form (ocDNA). The UV irradiation of DNA in the presence of H_2O_2 (lane b) resulted in the cleavage of sc DNA to ocDNA and linear form (linDNA), indicating that OH generated from UV photolysis of H_2O_2 produced DNA strand scission. The addition of PE (lane d to lane f) at different concentration to the reaction mixture of H_2O_2 suppressed the formation of linear DNA and induced a partial recovery of scDNA. The action of these natural compounds was comparable to 2 mM curcumin (c). The free radical scavenging capacity of these natural compounds was confirmed by protection against DNA strand scission, induced by OH radicals, generated from UV-photolysis of H_2O_2. In fact, these extracts suppressed the formation of linDNA and induced a Partial recovery of scDNA.

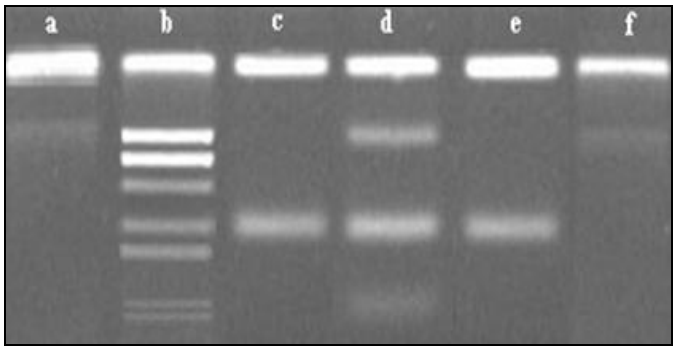

FIG. 2: ELECTROPHORETIC PATTERN OF DNA OBSERVED UNDER GEL DOC

a: without exposure to UV & H_2O_2; b: after exposure to UV & H_2O_2; C: curcumin (2mM); d: PE (300 µg/ml); e PE (400 µg/ml); PE (500 µg/ml)

Although both O_2 and H_2O are potentially cytotoxic, most of the oxidative damage in biological systems is caused by the OH^- radical, which is generated by the reaction between O_2 and H_2O_2 in the presence of transition metal ions. In fact, the OH^- radical can react with a number of target molecules including proteins, membrane lipids, and DNA. OH^- bound onto the DNA will lead to strand breakage, deoxysugar fragmentation and base modification.

Gelling and Bio-Adhesive Properties Evaluation: For the pharmacological assessment of the prepared formulation gelation, gel melting temperatures, as well as bio-adhesive strength (measured on goat colon), were initially examined. Pluronic, a block copolymer shows a thermoreversible gel at a concentration above 18% w/w. When the polymer solution is warmed, the PEO strands of neighboring rattles become entangled, resulting in a gel with a cubic structure. Gelation and gel melting temperature are shown in **Fig. 3** for the concentration range of 15-25%. Gelation temperature tends to decrease with increasing Pluronic F 127 concentration whereas gel melting temperature was found to increase with Pluronic F 127 concentration showing an inverse relationship.

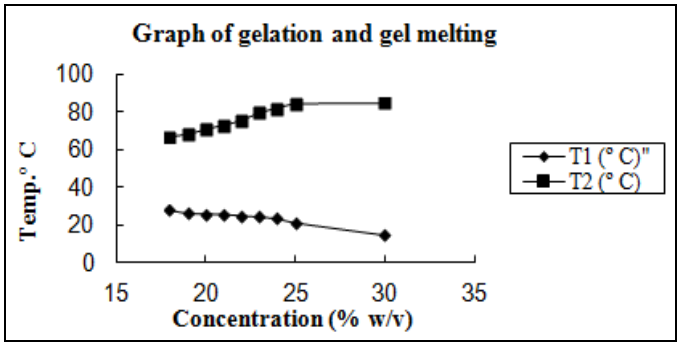

FIG. 3: EFFECT OF POLOXAMER CONCENTRATION ON GELATION AND GEL MELTING

Effect of PE addition on sol - gel transition and on gel - sol transition is shown in **Fig. 3**. Incorporation of *Porphyra* extract shifted sol - gel transition to lower whereas gel - sol to the higher temperature. Physically, gel formation is related to ratllar packing and volume fraction. Researchers have attributed gelation to the dehydration of PPO groups in the ratlle core, a change in the ratllar volume or a decrease in the critical ratlle concentration and increase in the aggregation number [18].

This feature was tentatively explained by facilitation of the interaction between the hydrophobic portion of the polymer in gelation temperature by vitamin B_{12} at all concentration of pluronic F127, attributed to the higher water solubility of vitamin B_{12}. The presence of the vitamin B_{12} predominantly decreased the critical ratllar concentration accompanied by increase ratllar aggregation [19]. PE has also shown an increase in gel melting temperature.

The entanglement of the large size molecule in outer PEO chain favoring hydration may be the factor responsible for an increase in melting temperature.

The bioadhesive strength of prepared gel was measured on Goat colon. The force of adhesion and work of adhesion shear (area under the curve) were taken as a parameter for mucoadhesion **Table 1**.

TABLE 1: BIOADHESIVE STRENGTH OF DIFFERENT BATCHES

Gel concentration (% w/v)	Max. force of Adhesion (in mN)	Avg. force of Adhesion (in mN)	Distance of Detachment (in mm)	Work of Adhesion shear (AUC) (mN.mm)
Blank gel				
18%	814 ± 6.4	534.41 ± 4.6	2.17 ± 0.41	1010.18 ± 8.1
20%	1026 ± 4.2	686.23 ± 5.1	3.45 ± 0.62	1870.04 ± 7.6
22%	943 ± 3.8	480.21 ± 2.6	3.68 ± 0.46	1490.27 ± 3.2
Drug-loaded gel				
18%	1240 ± 7.4	744.81 ± 4.7	3.04 ± 0.33	2460.19 ± 3.6
20%	1885 ± 6.6	768.21 ± 3.5	3.87 ± 0.43	2857.13 ± 8.5
22%	2490 ± 7.1	984.11 ± 5.2	4.10 ± 0.56	3841.11 ± 7.4

Macroscopic and Histopathological Evaluation: To study the effect of the prepared formulation of methanolic extract of *P. vietnamensis* for targeted local drug delivery to the inflamed tissue of colon in IBD, trinitrobenzene sulfonic acid (TNBS) induced experimental colitis model was selected. Moreover, it is the most relevant model as it involves the use of immunological haptens and develops a chronic inflammation rather than an acute mucosal injury. Intracolonic administration of TNBS results in ulcerative damage to the distal colon. Macroscopical observation reveals mucosal congestion, erosions and hemorrhagic ulcerations in the region of caecum, colon, and rectum. The histopathological analysis showed infiltration of leucocytes, necrosis, and edema in the submucosa.

Animals treated with rectal gel containing *P. vietnamensis* (0.5 ml daily containing 1000 mg per kg body weight) showed a significant reduction in the ulceration of the large intestine as shown by macroscopic damage score is shown in **Table 2** as well as histopathological assessment in **Fig. 4**.

After treatment with *P. vietnamensis* formulation, P-334, and standard mesalamine formulation there was a significant increase in body weight whereas in case of animals treated with only TNBS showed a loss in body weight. It was also observed that there was a significant increase in colon weight after treatment with TNBS (260.63 mg cm^{-1}) but after *P. vietnamensis* (204.75 mg cm^{-1}) and mesalamine (162.20 mg cm^{-1}) treatment it was reduced significantly. The colon weight for the healthy animal group was 112.68 mg cm^{-1}. Administration of PE and P-334 resulted in a significant reduction in the extent of damage and the incidence of adherence to adjacent organs whereas mesalamine did not affect the adherence to adjacent organs **Table 2**.

TABLE 2: EFFECTS OF DIFFERENT TREATMENTS ON DAMAGE SCORE, EXTENSION OF LESION, CHANGES IN COLON WEIGHT AND ADHERENCES TO ADJACENT ORGANS

Group name	Score rate [a]						N	Mean	Colon weight [b] (mg cm^{-1})	The extent of damage [c] (cm)	Ad. (%)[d]
	0	1	2	3	4	5					
HC	4	2	0	0	0	0	6	0.53	112.68 ± 6.28	0**	0**
CC	0	0	1	1	4	0	6	3.91	260.63 ± 37.62	3.67 ± 0.21	47
TC	0	4	1	1	0	0	6	2.11	204.75 ± 22.81	1.81 ± 0.14	25
PC	0	1	1	2	2	0	6	2.62	162.20 ± 8.14	1.34 ± 0.22	39
P-334	0	1	2	1	2	0	6	2.46	165.31 ± 7.61	1.37 ± 0.17	23

[a]Macroscopic score was analyzed Ukil *et al*., 2003; [b,c] Colonic weight and extent of damage data are expressed as mean ± SEM values and differences were assessed using ANOVA; [d]Adherence was expressed in percentage; HC: Healthy control; CC: colitis control; TC: Test control; PC: Positive control, P-334: Purified Porphyra-334; n: number of animals per group, *n: number of rats per group.

Above all these observations suggests the healing stage after colonic injury. The data obtained above was subjected to statistical treatment and p values were obtained. The p-value obtained was <0.001 for PE, and standard groups were considered extremely significant. Hence, results suggested that animals treated with *P. vietnamensis* gel showed recovery concerning the standard group.

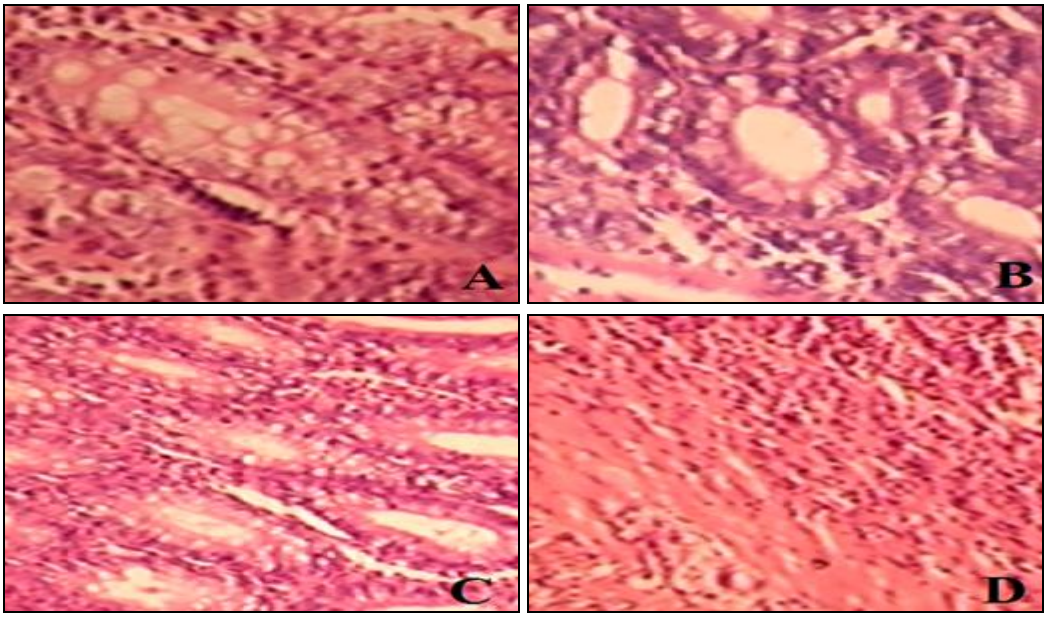

FIG. 4: HISTOPATHOLOGICAL FEATURES OF COLON SECTION. A: TEST CONTROL (RAT WITH TNBS + PE TREATMENT), B: STANDARD (RAT WITH TNBS+MESALAMINE TREATMENT), C: HEALTHY CONTROL, D: COLITIS CONTROL (RAT WITH TNBS TREATMENT)

In the case of animals from the healthy group showed normal architecture which is highlighted with evenly spaced crypts and keeping down up to muscular. There were no infiltration of lymphocytes, plasma cells, and eosinophils with Lamina propria. Further, in case of negative control histopathological observation suggested deep ulceration characterized by distorted architecture, severe infiltration of eosinophils and neutrophils, further lamina propria showed edema.

In positive control, histopathological analysis showed normal architecture but with minor distorted structure. Further, there was mild congestion and edema; lamina propria showed infiltration by neutrophils and eosinophils. Whereas *P. vietnamensis* treated group showed normal crypts, lamina propria showed a usual number of mononuclear inflammatory cells. These all histopathological observations suggested the potential of methanolic extract of *P. vietnamensis* in the treatment of IBD.

Colonic injury by TNBS administration was also characterized by an increase in malondialdehyde activity indicative of neutrophils infiltration in inflamed tissue confirming the enhanced leucocytes infiltration seen at histopathological inspection. In this study, the extent of myeloperoxidase activity closely paralleled the increase of tissue malondialdehyde indicative of massive lipid peroxidation.

Colonic injury by TNBS administration was also characterized by an increase in MDLA level that is indicative of massive lipid peroxidation (data shown in **Table 3**). However, treatment with PE and P-334 significantly prevented increase accumulation of malondialdehyde. The data obtained in the lipid peroxidation assay was statistically analyzed by Unpaired T-test. P value is <0.001; nevertheless *P. vietnamensis* did not produce any significant change in the elevated level of malondialdehyde when compared to the positive control.

TABLE 3: EFFECT OF *P. VIETNAMENSIS* GEL ON MALONDIALDEHYDE LEVELS IN TNBS-INDUCED IBD

Lipid peroxidation in rat intestine	Healthy control	Positive control	Negative control	PE	P-334
1	9.55	15.44	20.16	16.06	15.77
2	10.11	15.13	24.88	17.01	15.21
3	8.11	16.75	23.34	16.22	17.24
4	11..22	15.22	25.16	17.05	14.28
5	10.11	17.12	20.88	16.12	15.21
6	8.46	14.81	22.09	17.42	15.13
AVG	9.268	15.745	22.75167	16.64667	15.47333
STDV	0.934248	0.950973	2.065608	0.582466	0.988912

PE: *Porphyra* methanolic extract, P-334: Porphyra-334

Assessment of these physiological parameters put the *P. vietnamensis* as a strong candidature for Treatment in IBD due to its strong potential as antioxidant, anti-lipid peroxidation and anti-inflammatory.

It was suggested from previous research articles that herbals with good antioxidant and anti-inflammatory activity fail to be effective in the treatment of IBD after given by oral route. Treatment of IBD or distal colon may demand change in route of administration, so in current research article, we have tried topical application of *P. vietnamensis*.

The results obtained in the present study indicate that the methanolic extract of *P. vietnamensis* is a potential source of natural antioxidant and could, therefore, be exploited for its medicinal properties. Further formulating into the rectal gel and *in-vivo* screening for colitis demonstrated the beneficial role of *P. vietnamensis* extract. Above all observations, it can be concluded that IBD could be successfully treated with *P. vietnamensis* with judicious formulation approach.

CONCLUSION: *Porphyra* extract (500 µg/ml) showed significant effects in reducing colitis when compared with mycosporine-like amino acid (P-334) and Mesalamine. In conclusion, our results suggest a beneficial therapeutic activity for *Porphyra vietnamensis* extract as an anti-inflammatory medicinal plant for IBD conditions. This reinforces the use of this plant as a remedy for IBD conditions and the therapy prevention of recurrence, in traditional medicine. More studies are strongly recommended to establish the mechanisms that are involved in its beneficial pharmacologic actions.

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