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Research Article

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Innovative Rectal Suspension of Mesalamine Utilizing Mesoporous Silica for Sustained and Colon-Specific Drug Delivery

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Abstract

Mesalamine is an anti-inflammatory drug for the treatment of ulcerative colitis-related colon cancer. The present study aims to evaluate the rectal suspension of mesoporous mesalamine for improved localized effect of the drug in the ulcerative colon of rodents. Syloid 244FP and Syloid XDP were employed to form a solid dispersion of mesalamine using the solvent evaporation technique, with a drug-to-carrier ratio of 1:1. The formulations were evaluated for drug loading, and an in vitro drug release study was conducted over 8 hours. The drug and excipients were subjected to studies on incompatibility, surface characteristics, and surface morphology. A reconstitutable suspension (9% w/w) was prepared using carbomer 934 and Xanthum gum as suspending agents. Ulceration was induced in the animals, and the effect of the formulations on the ulcerative colon was observed through a histopathological study. The FTIR study revealed the compatibility between the drug and the carriers, while surface morphology and surface characteristics studies revealed the amorphization of the drug. Syloid 244FP was found to be the best carrier in terms of loading mesalamine and releasing the drug in a sustained manner for 8 hours. The histopathological study revealed that prolonged localization of the drug could provide significant healing in the ulcerative colons of the animals. Therefore, it can be concluded that the sustained release solid dispersion of mesalamine in mesoporous silica could enhance the local activity of mesalamine in the treatment of ulcerative colitis.

Keywords: Mesalamine; Rectal suspension; Mesoporous silica; Solid dispersion.

1. Introduction

Mesoporous silica, a type of silica characterized by its highly porous structure, has become a significant focus in pharmaceutical research as a potential drug delivery system. Its distinctive attributes, including a high surface area, adjustable pore dimensions, excellent stability, and the ability to encapsulate a variety of substances such as drugs, proteins, and biomolecules, make it an ideal choice as a carrier. Additionally, its excellent biocompatibility has been acknowledged by the FDA, highlighting its suitability for medical applications [1].

The controlled porous morphology of mesoporous silica offers unique interactions, charges, and capillary forces that enhance the quality of tablets, powders, granules, and capsules. The availability of a large surface area and pore volume in mesoporous silica enables active drugs to easily reside in the pores, facilitating the formulation of a monodisperse system [2]. They are highly effective in

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amorphizing the drug by entrapping it in their nanosized pores [3]. They also alter the surface chemistry, which impacts drug loading and drug release [4].

Solid dispersion, the dispersion of drugs in an amorphous matrix, has been considered an efficient method for improving the drug solubility and dissolution rate. This approach is widely adopted due to its simplicity in production and reproducibility [5].

During the process, drug molecules are uniformly distributed within a carrier, transitioning from a crystalline to an amorphous state through interactions with the carrier, thereby improving solubility and dissolution [6]. In addition to amorphization, particle size also plays a crucial role in the solid dispersion of drugs, thereby improving solubility. This method can be effectively implemented in the current study by incorporating mesoporous silica [7].

Mesalamine or mesalazine, a BCS IV drug, is a derivative of salicylic acid used as a first-line treatment for anti-inflammatory effects in colon-related diseases like ulcerative colitis and Crohn's disease. The mechanism of action of mesalamine is not completely understood. However, it is believed to exert a topical effect at the inflammation site through direct interaction with the damaged cells at the diseased site. The oral bioavailability is only 28% [8].

The colon-specific drug delivery system has gained significant attention for its effectiveness in treating inflammatory bowel disease, where a high local concentration of the drug can reduce systemic side effects and enhance therapeutic efficacy [9]. This fact necessitates the enhancement of mesalamine's dissolution to increase its localization at the site of action. This study aims to investigate the use of various grades of mesoporous silica to develop amorphous solid dispersions of mesalamine. These dispersions are designed to facilitate the formulation of a reconstituted suspension for rectal administration, thereby enhancing the efficacy of mesalamine in treating inflammatory conditions of the colon.

2. Materials and Methods

2.1. Materials

Hetero Labs Limited, Hyderabad, gifted the drug mesalamine. Carriers such as Syloid 244FP and Syloid XDP were obtained from Grace Pharmaceuticals, Mumbai. The remaining chemicals used in the formulation were of analytical grade and were used in the experiment as received from SD Fine Chemicals, Bengaluru.

2.1.1. Compatibility study by Fourier Transform Infrared (FTIR)

A compatibility study was conducted between different grades of mesoporous silica and the drug using the ATR technique with a Bruker model alpha II instrument at an ambient temperature of 25±0.5°C on a zinc selenide crystal plate. Before analysis, the sample was stored at room temperature at a relative humidity of 60 ± 5%. The spectrum was generated in the range 4000-400cm^{-1} [10].

2.2. Preparation of Amorphous solid dispersion of mesalamine with mesoporous silica

2.2.1. Calculation for the quantity of drug and mesoporous silica for drug loading

Mesoporous silicas are novel carriers for drugs, featuring a solid network of porous structure and a larger surface area, which provides high pore volume to entrap drugs in their mesopores. The drug loading in mesoporous silica occurs by the adsorption of the drug on its active surface. Adsorption occurs in different layers and is significantly influenced by the method of preparation and the duration of incubation. The extent of drug loading in the monolayer can be calculated by the Denning and Taylor equation [3].

$$Theoritical\ drug\ load\ at\ monolayer\ adsorption\ \left(\%\frac{g}{g}\right) = \frac{SSA \times M_W \times 10^{20}}{SA_M \times N_A}$$

SSA: Specific surface area of mesoporous silica in (m^2/g)
Mw: Molecular weight of model drug in g/mol
SAM: Maximum projected contact surface area of a single molecule
N_A: Avogadro's number (6.022×10^{23}).

In the preparation of amorphous solid dispersion of mesalamine, two different grades of mesoporous silica (Syloid 244FP and Syloid XDP) were used as carriers. The solvent evaporation method was used to prepare the solid dispersion. The quantity of drug and carrier was calculated as per the Denning and Taylor equation [11].

Hence, in the present study, a molar ratio of 1:1 carrier and drug was taken for the studies, considering the maximum drug loading. Two formulations (TSFP and TXDP) were prepared using Syloid 244FP and Syloid XDP.

The carrier and the drug were dispersed in ethanol (5% w/v) to form a slurry. The dispersion was incubated in a mechanical shaker for 24 h at room temperature. Later, the dispersion was filtered, and the residual solvent was evaporated using a rotary evaporator at 50°C and 100 rpm [11]. The solid dispersion thus obtained was collected, air-dried, and evaluated for further studies.

2.3. Estimation of drug content

The drug content was estimated by dispersing a known quantity (5 mg) of the sample in a phosphate buffer solution (10 mL), pH 6.8. The solution was diluted to a suitable concentration, and its concentration was estimated spectrophotometrically using UV spectroscopy at 330 nm. The standard absorbance of the pure drug (5 mg) was estimated using the same dilution and compared with the sample of standard concentration to determine the drug content. All formulations were tested, and three trials were conducted for each formulation [12, 13].

$$\%\ Drug\ content = \frac{Absorbance}{Standard\ Absorbance} \times 100$$

2.4. Estimation of drug loading

Drug loading in the mesopores of silica was estimated by the determination of free or unentrapped drug by the UV spectroscopy method (UV-1900i, Shimadzu). The entire quantity of the formulation, immediately after preparation, was filtered using Whatman filter paper grade 42. The filtrate was then centrifuged, and the supernatant was collected and diluted suitably with phosphate buffer solution (pH 6.8). The absorbance was recorded at 330 nm. The estimation was carried out in triplicate. The average absorbance was taken to determine the presence of the unentrapped drug. The percentage drug loading was calculated using the following formula.

$$\%Drug\ loading = \frac{Total\ drug\ in\ formulation - free\ drug}{Total\ drug\ in\ formulation} \times 100$$

2.5. In-vitro drug release study

In vitro dissolution was performed according to the USP procedure for extended-release dosage forms using a USP DS8000 (Lab India) apparatus type II. A quantity of 400 mg equivalent sample was filled into a hard gelatin capsule, and dissolution was carried out for 480 min at 100 rpm at 37 °C ± 1 °C in 900 mL of phosphate buffer (pH 7.5) [14]. In each phase, 1 ml of sample was withdrawn at regular intervals and replaced with fresh medium. The experiment was performed in triplicate for the pure drug and the formulations. The in vitro drug release data were expressed as mean ± standard deviation from three independent replicates.

2.6. Statistical analysis

To compare the release profiles statistically, Dunnett's test was employed, using the pure drug as the control group. This test allowed for a point-to-point comparison between the test formulations (TSFP and TXDP) and the pure drug, while adjusting for multiple comparisons to minimize Type I error. A significance level of $p < 0.05$ was considered statistically significant, enabling the identification of any significant differences in drug release behavior. Dunnet's test was conducted to determine the optimal formulation for further studies [15].

2.7. Powder X-Ray diffraction (PXRD) study

PXRD was performed for the pure drug and the best formulation. PXRD was performed using a Bruker D8 Advance (Bruker Germany) diffractometer at room temperature. A small sample was analyzed at a voltage of 40 kV and a current of 30 mA using a LYNXEYE (1D) detector. 2Θ was scanned for the 4°to 60°at rate of 0.02°/min 2Θ [2, 3].

2.8. Scanning electron microscopy (SEM)

SEM was performed for the pure drug, Syloid 244FP, and the best formulation. SEM was performed on a JSM-IT300 (Joel, Japan) instrument at IISC Bangalore. A small quantity of powder drug was mounted on the stubs using black thin adhesive tape, forming a layer of powder on it. A working distance of 14 mm with a voltage of 15 kV was applied for scanning the sample, and a magnification of x2500 was used.

2.9. Preparation and evaluation of Rectal suspension

Reconstituted rectal suspension of mesalamine was prepared by mixing TSFP (9%w/w), Carbomer 934 (0.5% w/w), and Xanthum Gum (0.5% w/w). The powder mixture was reconstituted with 50 mL of water. The prepared rectal suspension was evaluated using a pH meter.

2.10. Histopathology study

An animal study was conducted using albino rats of either sex, weighing 200-250 g. The permission for the use of animals in the study was obtained from the Institutional Animal Ethics Committee (IAEC) vide approval KCP/IAEC/PCOL/PCEU/83/2022. The animals were divided into four groups, each group consisting of six animals. Group I animals served as controls. An ulcer was induced in the animals of groups II, III, and IV using 5% v/v acetic acid in saline (0.5 mL/rat) through the rectal route under mild anesthesia. The animals were allowed to recover from mild anesthesia, and food and water were given ad libitum. The animals were observed for 3 to 4 days. The presence of watery stool confirmed the development of ulcers. Group II animals were not subjected to any treatment. Group III and IV animals were treated with a suspension of the pure drug and the best formulation, respectively. The formulation was dispersed in sterile water for injection, warmed to 37℃, and administered rectally in the presence of mild anesthesia with a calculated dose of 4.5 mg per day [16]. The animals were fasted for 12 h before treatment, with water available ad libitum. After recovering from anesthesia, they were provided with food and water.

Colonic damage and recovery were studied in groups II, III, and IV over 4 days. The animals of all the groups were sacrificed, and 7 cm of the colon from the anal orifice of the animals was collected to compare the healing effect of the formulation.

The colonic specimen was fixed in 10% formalin phosphate buffer solution (pH 7.4) before embedding, sectioning, and staining with hematoxylin. Histopathological studies of the induced and normal colon were carried out at VEMSA BIO-TECH Pvt Ltd (Bangalore) using an LB-239 digital microscope with an attached camera [17].

3. Results and Discussion

3.1. Compatibility study by FTIR

FTIR spectra of the drug with silica were carried out, using Bruker model alpha II, and are shown in **Figure 1**. The major peaks of the pure drug identified are O-H stretch 3490nm^{-1}, N-H bending 1617nm^{-1}, O-H bending 1447nm^{-1}, C-H bending 1353 nm^{-1}, in-plane bending 1264nm^{-1}, CH bond out of plane bending 685nm^{-1}. The spectra obtained for the physical mixtures of the drug with different grades of silica (Syloid 244FP and Syloid XDP) indicate no significant chemical interaction between the drug and carrier in the formulation, and all the characteristic bands of the pure drug were preserved. Therefore, the compatibility of the drug with different grades of mesoporous silica is established.

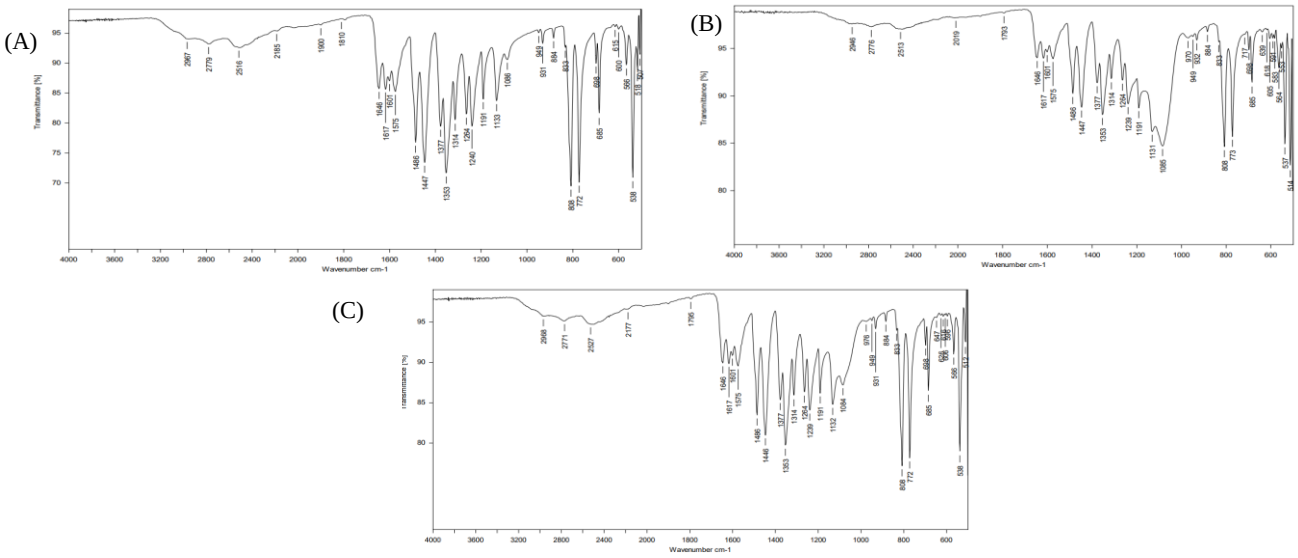

Figure 1. FTIR spectra of Pure drug (A), TSFP (B), TXDP(C).

3.2. Evaluation of the solid dispersion

The drug content and loading of the formulations were estimated. TSFP has a drug content of 85 ± 0.89%**,** slightly higher than TXDP's 83 ± 1.05%**.** The narrow standard deviation for both formulations indicates a consistent and uniform distribution of the drug within the formulation. TSFP shows a drug loading of 76 ± 1.21%**,** which is higher than TXDP's 70 ± 3.15%**.** TSFP also demonstrates less variability in drug loading compared to TXDP, as evident from its smaller standard deviation. This suggests that TSFP might offer better control over the incorporation of the active pharmaceutical ingredient (API) into the formulation.

The results further indicated the suitability of process parameters, including the selection of the solvent system and incubation time, for preparing solid dispersions. Furthermore, the drug loading suggests successful adsorption of the drug onto the pores of silica.

3.3. In vitro drug release study

Since the final dosage form is a suspension, a quantity of the suspension equivalent to 400 mg of the mesalamine dispersion was filled into hard gelatin capsules for in vitro evaluation. A 400 mg equivalent was chosen as a representative sub-dose for dissolution profiling, facilitating manageable in vitro testing volumes. This approach was adopted to ensure accurate and consistent dosing, facilitate standardized testing conditions, and improve reproducibility across replicates. The capsules were designed to rapidly disintegrate upon contact with the dissolution medium, thereby releasing the dispersion without altering its inherent characteristics of drug release. An in vitro release study was conducted for formulations TSFP and TXDP, and compared with the pure drug over 8 hours. The percentage of cumulative drug release vs. time is depicted in **Figure 2**. The data indicate that both formulations demonstrated an enhanced drug release profile compared to the pure drug. This enhancement can be attributed to the unique formulation characteristics. Both TSFP and TXDP exhibited a sustained release pattern, maintaining a steady-state drug release throughout the study period. This consistent release can be explained by the "spring and parachute effect," wherein the amorphous drug form (spring) ensures rapid initial solubility, followed by a slower release due to the "parachute effect."

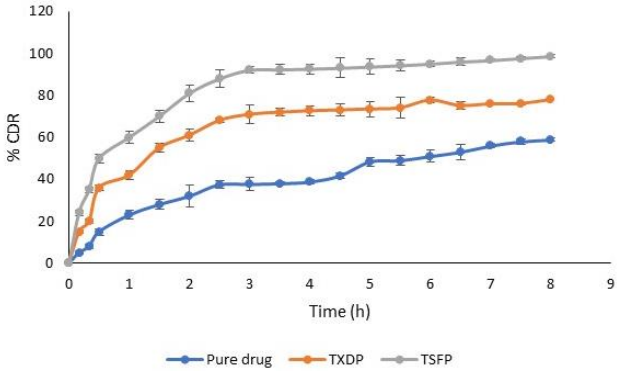

Figure 2. *In vitro* drug release study of the formulations

In contrast, the porous silica matrix (parachute) modulates and sustains the release rate [18, 19]. The initial rapid release observed is likely due to the diffusion of dissolution media through the interconnected empty channels of the porous silica particles, facilitating immediate drug release. The subsequent steady-state release phase could be governed by a combination of diffusion and dissolution-controlled mechanisms, ensuring prolonged drug availability [20]. The dissolution parameters were calculated and listed in **Table 1**.

Table 1: Comparison of dissolution parameters

Dissolution Parameters	Pure Drug	TXDP	TSFP
AUC	317.85	521.12	623.15
MRT (h)	3.48	3.12	1.75
MDT (h)	2.60	1.32	1.12
DE (%)	40	65	83

The formulation TSFP has the highest AUC value, followed by TXDP and the pure drug; this indicates overall drug release within the stipulated time, which may demonstrate more prolonged localization of the drug at the site. A shorter MRT for TSFP indicates faster drug release compared to TXDP and the pure drug, which may enhance the onset of action, while a lower MDT for TSFP and TXDP suggests faster dissolution. A higher DE indicates improved efficiency in drug dissolution, which translates to better localization and therapeutic performance for TSFP and TXDP compared to the pure drug.

3.4. Powder X-ray diffraction (PXRD)

The PXRD of pure drug and formulations (TSFP and TXDP) are shown in **Figure 3**. The PXRD of mesalamine and its formulations (TSFP and TXDP) was performed to

study the characteristics of mesalamine and mesalamine loaded in the mesoporous carrier. The pure drug exhibited high-intensity peaks, whereas in the formulations, the high-intensity peaks of the pure drug were significantly reduced. This might be the reason for the improvement in solubility and dissolution of the formulations. The PXRD pattern analysis is presented in **Table 2**.

Table 2. PXRD pattern analysis

2Θ	Pure drug	TSFP	TXDP
7.70	52317	7773	17037
16.49	11903	4677	5257
16.63	37235	7898	15229
24.32	8846	6459	8846
27.27	29305	10739	13429
28.30	19366	7014	8739
30.66	8776	2312	3610
36.70	7270	2666	3141
38.59	6396	2426	2884
40.52	6054	1916	2599
43.69	7690	2123	3278
46.42	1226	993	988
49.75	1502	1105	959
49.80	1707	1117	1059
53.32	1346	899	943
55.55	1242	938	820

3.5. Scanning Electron Microscopy (SEM)

SEM analysis was conducted to analyze the morphology characteristics of the solid dispersions. The SEM photographs of pure drug, Syloid 244FP, and TSFP are shown in **Figure 4**. SEM images of mesalamine confirmed its crystalline nature, characterized by well-defined structures. At higher magnification, the carrier materials exhibited distinct channel-like architectures. In contrast, the TSFP formulations displayed nanosized solid dispersions, as evidenced by the images, which showed a fine dispersion lacking distinguishable ~~pure~~ components. The formulations appeared as smooth-surfaced particles with partial agglomeration. The absence of crystalline structures and the presence of uniformly distributed fine particles within the carrier matrix provide strong evidence for the formation of a solid amorphous mass.

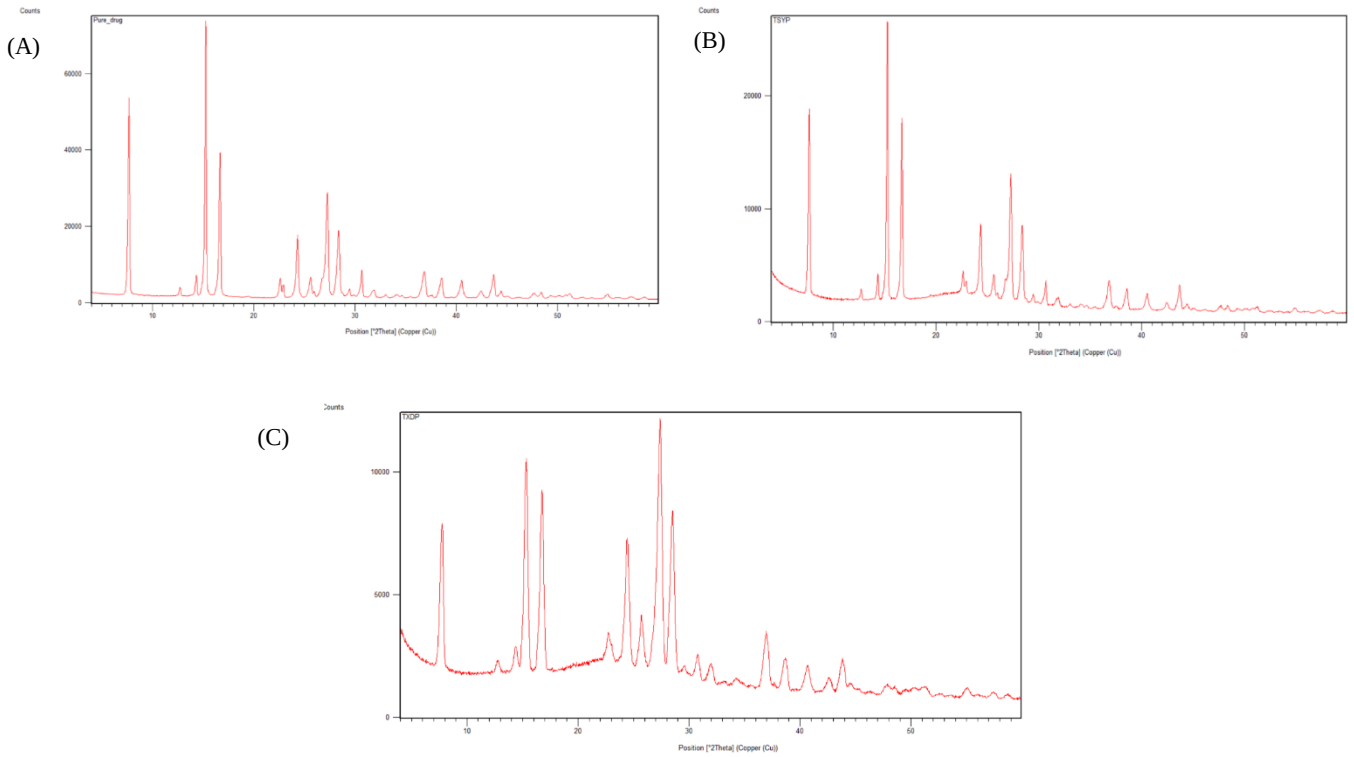

Figure 3. PXRD Pattern of Pure drug(A), TSFP(B) and TXDP(C).

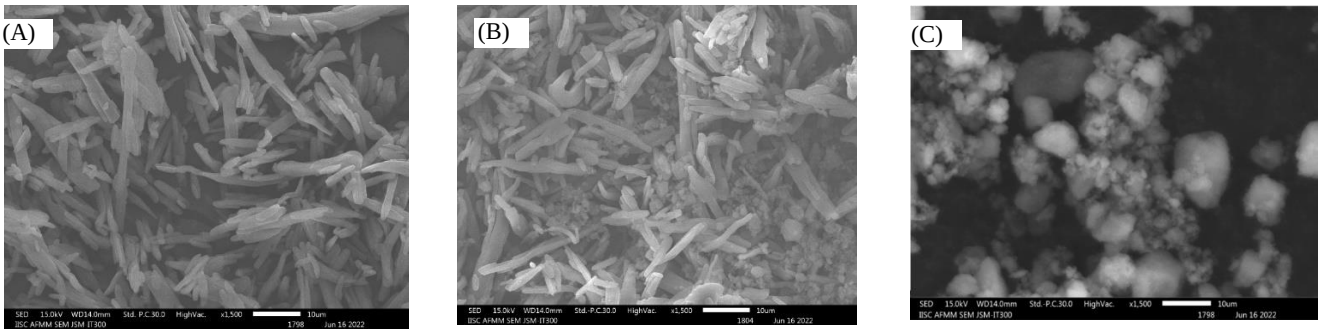

Figure 4. SEM Images of Pure drug(A), Syloid 244FP(B) and TSFP(C).

3.6. Histopathology study

The formulation TSFP was subjected to an animal study to assess its ulcerogenic activity. The formulation subjected has shown a potential decrease. The ulcer was localized in the colon, as shown in **Figure 5**. The result was confirmed by the histopathologic report taken from each group of albino rats. The results were based on the scoring of the ulcer, where zero indicates normal and 5 indicates the most damaged. The histopathology report of group (a) shows a normal, healthy colon of the rat with a score of 0. Group (b) shows the necrosis of the mucosa and submucosa, as well as hemorrhage in rats treated with acetic acid, with a score of 5+. Group (c) shows the effect of the pure drug mesalamine on the colon of the rat at 24 h with a score of 2+, and group (d) shows the effect of TSFP on the animals at 24 h (1+).

The effect of mesalamine was found to be effective within 6 hours, and efficacy was observed to last for 24 hours. This proves the sustained effect of the solid dispersion. Hence, the histopathology report confirmed the anti-ulcer action of the formula.

4. Conclusion

The study successfully demonstrates the potential of mesoporous silica-based solid dispersion in enhancing the therapeutic efficacy of mesalamine for the treatment of ulcerative colitis in rodents. Mesoporous silicas, particularly Syloid 244FP, proved to be effective carriers due to their superior drug-loading capacity, compatibility with mesalamine, and ability to form an amorphous drug state. Statistical analyses and histopathological evaluations confirmed that the Syloid 244FP-based formulation significantly improved healing in ulcer-induced animal models, with prolonged drug localization and sustained anti-inflammatory action. Overall, the innovative use of mesoporous silica in this study highlights its promising role in the development of effective colon-targeted drug delivery systems. Future studies could explore its scalability and clinical application, paving the way for advanced treatments in inflammatory bowel diseases.

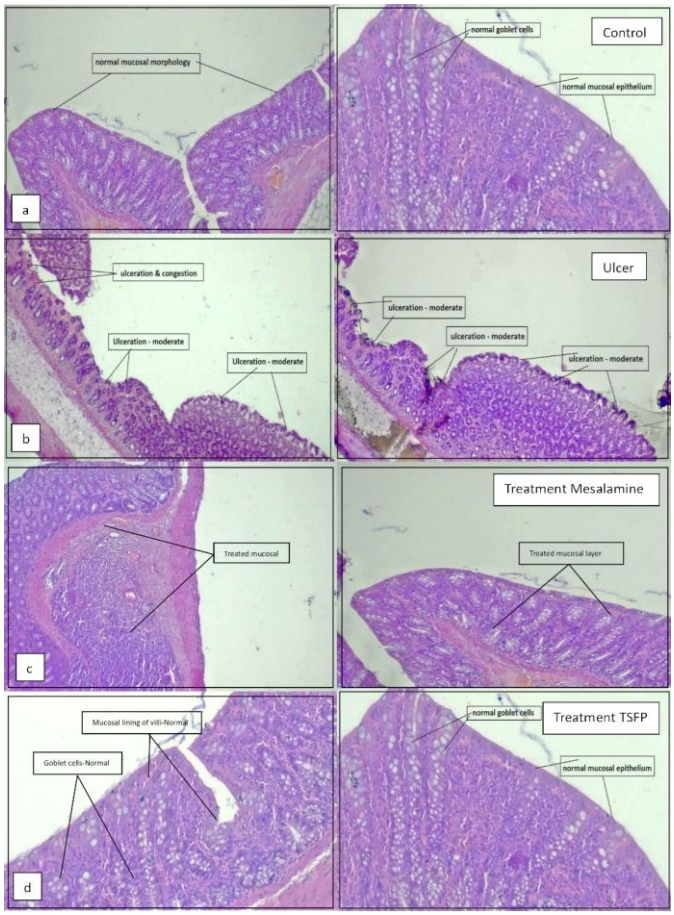

Fig.5 Histopathology of the colon of rodents
(a) Control – Normal Mice Colon showing – normal mucosal epithelial morphology -NAD^+ (X50)
(b) Ulcer induced – Mice Colon showing – ulceration with congestion – moderate - 3+ (X50)
(c) Treatment (mesalamine) -Mice colon showing repaired colitis in the mucosa
(d) Treatment with TSFP - Mice colon after 24

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Conflict of interest

The authors declare no conflict of interest.

Data availability

The dataset of the current study is available upon request.

Athors Contributions

Concept, Design, Manuscript writing-SB; Conduct of experiments, Manuscript writing-RUP; Manuscript writing, revision-UB.

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

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