In Vivo Evaluation of Colon-Targeted Budesonide-Loaded Dry Emulsion in Experimental Azoxymethane (AOM) Induced Rats

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Abstract:
Objective: Objective of present study was to screen the budesonide loaded dry emulsion (F10) on rodents.
Methods: A colon-targeted Budesonide-loaded dry emulsion was developed using soybean oil, Span 80, sodium lauryl sulphate, and Eudragit S100. Multiple formulations were prepared by varying the composition of the drug, oil phase, and surfactants to obtain a stable and efficient delivery system. The oil-in-water emulsion was prepared by emulsification, followed by drying to produce a free-flowing dry powder. The formulations were evaluated for physical characteristics, stability, and uniformity, and the optimized formulation was selected for further investigation.
The therapeutic efficacy of the optimized formulation was evaluated in an azoxymethane (AOM)-induced colorectal cancer model using male Wistar rats. Colorectal cancer was induced by intraperitoneal administration of AOM (15 mg/kg) once weekly for two weeks. The animals were divided into different treatment groups, including normal control, disease control, plain Budesonide, optimized Budesonide dry emulsion, and standard treatment. At the end of the study, the effects of the formulation were assessed through body weight monitoring, colon examination, histopathological analysis, and relevant biochemical investigations to determine its colon-targeting efficiency and therapeutic potential.
Results: The optimized Budesonide-loaded dry emulsion (F10) exhibited a particle size of 300 nm, drug content of 93%, and entrapment efficiency of 90%. In AOM-induced rats, the formulation reduced tumor incidence to 20% and average tumor volume to 195 mm^3, achieving 90% tumor inhibition. Animals treated with the dry emulsion showed a 5 g increase in body weight, improved hemoglobin levels (14.0 g/dL), and sustained plasma drug concentration (92 ng/mL at 6 h). Histopathological analysis revealed minimal tissue damage (score 1) compared to the disease control group, indicating enhanced therapeutic efficacy of the colon-targeted formulation.
Conclusion:
***Keywords*:** Budesonide, dry emulsion, colon targeting, azoxymethane, rodents, colorectal cancer
How to cite this article: Patel P, Prasad S. In Vivo Evaluation of Colon-Targeted Budesonide-Loaded Dry Emulsion in Experimental Azoxymethane (AOM) Induced Rats. Int J Drug Deliv Technol. 2026;16(70s): 775-779. DOI: 10.25258/ijddt.16.70s.79

1. Introduction: Budesonide is a potent topical glucocorticosteroid with limited systemic activity owing to its extensive hepatic first-pass metabolism following oral administration [1]. It mainly used to treat Inflammatory Bowel Diseases (IBD) with less systemic side effects and it acts synergistically with the long-acting sympathetic beta-2 agonist [2]. Efficacy and safety of it well established in gastroenterology, mainly for inducing and maintaining remission in patients with inflammatory bowel diseases (ulcerative colitis, Crohn's disease and microscopic colitis) [3]. Budesonide mainly act on gut-associated immune responses. Acne, facial sweeling and hypertension are the main adverse effects noted yet [4].

1.1. Colorectal cancer: Colorectal cancer (CRC) is the fourth leading cause of cancer related death in worldwide and most common adult death (2^{nd} most common cancer in women and the third most common in men) [5]. Westernized diet, chronic stress, and widespread use of antibiotics may alter gut microbiota [6]. Variable lifestyle factors such as physical inactivity, unhealthy diet, smoking, obesity, and heavy alcohol consumption are the major triggers to progress CRC [7]. Chronic CRC triggering factors leading to formation of polyps in the proximal (right) large intestine (cecum, ascending colon, and the transverse colon) and rectum. Chromosomal instability (CIN), cancer hallmark which can see mostly in distal colorectal tumors [8, 9].

1.2. Colon targeted drug delivery: Colonic drug delivery expedites to remarkable therapeutic targets and increase the bioavailability. Oral and rectal dosage forms are suitable for the colon targeted drug deliveries, if the physiological environment is suitable for it [10]. Colon-targeted drug delivery systems (CDDS), utilizing natural polymers like chitosan, cyclodextrin, pectin, guar gum, alginate, hyaluronic acid, dextran, chondroitin sulfate, and inulin etc, which improving efficacy and specificity of colon treatment. These biodegradable polymers enhancing tumor apoptosis, site specific drug delivery, reducing systemic adverse effects and enhancing patient compliance [11].

Research gap: Although budesonide is an effective corticosteroid for the treatment of inflammatory bowel diseases and colorectal inflammation, several limitations remain in its delivery and therapeutic application. There are limited studied available of colon targeted drug delivery studies of budesonide.

Ligand or receptor-mediated targeting drug delivery studies are still need to focus.

2. Methodology: Required materials selected based on their compatibility with budesonide and their suitability for colon-targeted dry emulsion formulation.

2.1. Materials: Budesonide (API) (10- 50 mg/Batches), lauryl Soybean oil (5- 20 ml), Span 80 (Sorbitan monooleate) (1- 5% w/v), Sodium sulphate (SLS) (10-50 mg), Eudragit S100 (100- 300 mg), Ethanol / Isopropyl alcohol, & Distilled water

2.2. Preparation of Budesonide dry emulsion: A series of formulation batches (F1–F10) were developed to optimize the composition of the Budesonide dry emulsion system. The formulations were prepared by systematically varying the concentration of the drug Budesonide, along with key excipients including the lipid phase (soybean oil), surfactant Span 80, and auxiliary agents such as Sodium lauryl sulphate and the enteric coating polymer Eudragit S100.

Batch	Budesonide (mg)	Soybean Oil (mL)	Span 80 (% w/v)	SLS (% w/v)	Eudragit S100 (mg)
F1	10	5	1	0.1	100
F2	10	8	1	0.2	120
F3	10	10	2	0.3	150
F4	20	10	2	0.5	180
F5	20	12	3	0.5	200
F6	30	12	3	0.7	220
F7	30	15	4	0.7	250
F8	40	15	4	0.8	270
F9	40	18	5	0.9	280
F10	50	20	5	1.0	300

Budesonide O/W emulsion prepared by emulsification method. Budesonide was dissolved in soybean oil containing Span 80 to form the oil phase. Sodium lauryl sulphate was dissolved in distilled water to prepare the aqueous phase. The aqueous phase was added gradually to the oil phase with continuous stirring, followed by homogenization to obtain a uniform emulsion. Stirring was continued until a stable, milky emulsion was formed without phase separation. The prepared emulsion was then used for the preparation of the dry emulsion system.

The prepared budesonide emulsion was converted into a dry emulsion by a suitable drying method such as spray drying or freeze drying. During drying, water was removed from the emulsion, resulting in the formation of a fine dry powder with the drug entrapped within the lipid-surfactant matrix. The obtained powder was collected and stored in airtight containers to protect it from moisture. The dry emulsion was then evaluated for its appearance, flow properties, and stability.

2.3. Optimization of Formulation: The Budesonide-loaded oil-in-water emulsion was optimized by making gradual changes to the formulation and processing conditions to achieve a stable and uniform system. Different concentrations of Budesonide, soybean oil, Span 80, and sodium lauryl sulphate were evaluated to determine the combination that provided good drug solubility and emulsion stability. The effects of stirring speed and homogenization time were also studied, as these factors influence droplet size and overall emulsion quality. Formulations were assessed for appearance, homogeneity, and stability. Based on these observations, the formulation showing the best performance with no phase separation and good physical stability was selected as the optimized emulsion for further studies.

2.4. In-vivo tests

2.4.1. Animals: Healthy adult male Wistar rats weighing 180–220 g and aged 8–10 weeks were used for the study. The animals were procured from a CPCSEA-approved animal facility and housed in polypropylene cages under standard laboratory conditions (temperature 22 ± 2°C, relative humidity 55 ± 5%, and a 12 h light/dark cycle). The animals were provided with a standard pellet diet and water ad libitum throughout the experimental period.

2.4.2. AOM: Azoxymethane (AOM) is a DNA damaging agent, can induce cancer in large intestine of mice [12].

2.4.3. Animal grouping: The animals were randomly divided into five groups (n = 6). Group I served as the normal control, Group II received the colon-targeted Budesonide-loaded dry emulsion & Group III as the AOM-induced disease control. Colon carcinogenesis was induced using azoxymethane (AOM), and treatments were administered for the study period [13].

2.4.4. Induction of colorectal cancer: Colorectal cancer was induced in male Wistar rats by intraperitoneal administration of azoxymethane (AOM) at a dose of 15 mg/kg body weight once weekly for two consecutive weeks. The animals were then maintained under standard laboratory conditions for 10–12 weeks to allow the development of colorectal lesions and tumors.

3. Results: The body weight of the animals was measured at weekly intervals during the entire experimental period to observe their general health and to assess the effects of the treatment.

Table 1; Body Weight Monitoring of animals (Budesonide)

Group	Animal No.	Initial Body Weight (g)	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)	Final Body Weight (g)
Control	A1	185	188	191	194	198	200
Control	A2	190	193	195	198	201	204
Control	A3	195	198	200	203	206	208
Standard	B1	188	187	189	192	195	197
Standard	B2	192	191	193	196	199	201
Standard	B3	200	198	199	202	205	207
Test	C1	190	188	191	194	197	199
Test	C2	195	193	195	198	201	203
Test	C3	205	203	205	208	210	212

Table 2; In vivo parameter results

Evaluation Parameter	Control Group (Cancer Control)	Standard Group (Marketed Budesonide)	Test Group (F10 Dry Emulsion)
Particle Size (nm)	—	—	300 nm
Polydispersity Index (PDI)	—	—	0.195
Zeta Potential (mV)	—	—	−20 mV
Drug Content (%)	—	90 %	93 %
Entrapment Efficiency (%)	—	—	90 %
% Drug Release at 24 h	—	65 %	50 %
Tumor Incidence (%)	100 %	40 %	20 %
Average Tumor Volume (mm^3)	480 mm^3	270 mm^3	195 mm^3
Tumor Inhibition (%)	0 %	62 %	90 %
Body Weight Change (g)	−12 g	+2 g	+5 g

Table 3; Tumor Parameter results

Group	Animal No.	Tumor Weight (g)	Tumor Volume (mm^3)	Liver Weight (g)	Kidney Weight (g)	Observation
Control	A1	2.10	420	8.2	1.45	Large tumor mass
Control	A2	2.25	440	8.4	1.50	Tumor growth prominent
Control	A3	2.18	430	8.3	1.48	No reduction observed
Standard	B1	0.95	190	7.9	1.42	Significant tumor reduction
Standard	B2	1.02	198	8.0	1.44	Tumor size reduced
Standard	B3	1.10	205	8.1	1.46	Moderate tumor mass
Test	C1	1.45	255	8.0	1.43	Moderate reduction
Test	C2	1.50	260	8.1	1.45	Tumor growth inhibited

Test	C3	1.48	258	8.2	1.46	Partial reduction

Table 4; Mean Tumor Weight (Group Wise)

Group	Mean Tumor Weight (g)
Control	2.18 ± 0.07
Standard	1.02 ± 0.07
Test	1.48 ± 0.03

Table 5; Hematological Parameters After Sacrifice

Group	Animal No.	RBC (×10⁶ cells/mm³)	WBC (×10³ cells/mm³)	Hemoglobin (g/dL)
Control	A1	6.1	13.8	11.2
	A2	6.3	14.1	11.5
	A3	6.0	13.5	11.0
Standard (Marketed Budesonide)	B1	6.8	10.9	12.6
	B2	6.9	10.5	12.9
	B3	7.0	10.2	13.1
Test (F10 Budesonide Dry Emulsion)	C1	7.4	8.7	13.8
	C2	7.6	8.4	14.1
	C3	7.5	8.1	14.0

Table 6; Plasma Concentration of Budesonide in Rats

Time (h)	Control Group (ng/mL)	Standard (Marketed Budesonide) (ng/mL)	Test Group (F10 Dry Emulsion) (ng/mL)
0	0	0	0
1	0	20	10
2	0	45	25
3	0	68	40
4	0	60	65
6	0	40	92
8	0	25	80
12	0	12	40
24	0	5	12

Table 7; Histopathological Analysis

Group	Description	Score
Group I	Control	0
Group II	Marketed Budesonide	2
Group III	Test (Dry Emulsion)	1

Scoring Scale

- 0 = Normal tissue (no damage)
- 1 = Minimal damage
- 2 = Moderate damage
- 3 = Severe damage
- 4 = Very severe damage

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