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Preparation, Optimization And Evaluation Of Anti-Inflammatory, Analgesic And Inflammatory Bowel Disease Of Indomethacin Raft Forming Tablets

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ABSTRACT

This research focuses on developing a gastro-retentive floating tablet of Indomethacin, a non-steroidal anti-inflammatory drug (NSAID), using the raft-forming approach. The formulation incorporates Indomethacin as the active ingredient, with quercetin (a polyphenolic compound) and raft-forming polymers serving as adjuncts. Quercetin contributes additional anti-inflammatory and antioxidant properties, helping to protect against gastric damage. To optimize the raft-forming tablet, we employed a central composite design and response surface methodology using Design Expert® software (version 11.1.2.0). The experimental design incorporated three levels, two factors, and one process parameter. The experimental design utilized three levels, two factors, and one process parameter. Pectin (X1), Quercetin (X2), and wet granule thickness (X3) were selected as critical independent variables. Tablets were prepared using the wet granulation method and evaluated for weight variation, hardness, thickness, friability, drug content, floating lag time, and raft strength—all of which met standard specifications. The optimized formulation (F11) achieved a cumulative drug release of 89%. This formulation was further evaluated in vivo for anti-inflammatory activity using the carrageenan-induced rat paw edema model, analgesic efficacy via the tail-immersion test, and effectiveness in inflammatory bowel disease (IBD). Results demonstrated that the optimized raft-forming tablet significantly reduced paw edema compared to the inducer group. Analgesic testing showed that the formulation containing Quercetin produced a significant increase in reaction time ($p < 0.0001$) compared to the inducer group, with percentage inhibition comparable to standard Diclofenac sodium.

INTRODUCTION

Oral route of administration is frequently regarded as the most convenient and favored method for introducing drugs into the systemic circulation[1]. Patient adherence to oral dosage forms tends to be high, primarily due to their ease of administration and handling. Recent technological advancements have enabled the development of oral delivery systems capable of providing therapeutic effects for 24 hours or more for various medications. However, for optimal effectiveness, it is essential that the drug is adequately absorbed throughout the gastrointestinal tract (GIT)[2]. Gastro-retentive dosage forms are specifically engineered to remain in the gastric region for prolonged durations, which can significantly enhance the gastric retention time (GRT) of drugs[3]. The raft-forming system is the most commonly utilized approach, as it is one of the most practical and preferred methods for achieving a sustained and predictable drug delivery profile within the gastrointestinal tract. This system is designed to release a drug molecule in a controlled manner, resulting in relatively stable plasma concentration profiles[4].

Raft-forming systems have garnered considerable attention for their efficacy in delivering antacids and addressing gastrointestinal infections and disorders. The mechanism behind raft formation involves the development of a thick, cohesive gel upon contact with gastric fluids. As this gel expands, it creates a continuous layer referred to as a raft, which remains buoyant due to the carbon dioxide released during the process. This raft serves as a protective barrier, inhibiting the reflux of gastric contents, including hydrochloric acid and digestive enzymes, back into the esophagus. Typically, these systems comprise a gel-forming agent combined with alkaline bicarbonates or carbonates, which help decrease density and enable the raft to float on gastric fluids.[5]

The raft-forming technique utilized in floating drug delivery systems significantly enhances drug absorption within the stomach and boosts bioavailability. Furthermore, the buoyancy of these systems prolongs gastric retention time, which helps to reduce gastric irritation.[6] When these hydrogels interact with bodily fluids or undergo a pH change, they form a gel at room temperature. The design of this system aims to either reduce the frequency of dosing or enhance the medication's efficacy by targeting the site of action, thereby simplified administrationdecreases the required dosage or ensuring a consistent drug release.[7] The formulation of raft-forming agents incorporates indomethacin, quercetin, and agents such as guar gum and pectin, along with alkalizing agents like sodium bicarbonate. The primary goal of this research was to enhance patient compliance by increasing bioavailability and therapeutic efficacy while preventing gastric lesions associated with NSAID use, specifically indomethacin, through the incorporation of quercetin. Quercetin, a flavonol and potent antioxidant found in various foods such as onions, grapes, berries, cherries, broccoli, and citrus fruits, plays a crucial role in this formulation. It is recognized for its protective properties against tissue damage caused by different drugs. Antioxidants like quercetin function as radical scavengers, inhibit lipid peroxidation, and mitigate free radical-mediated damage, leading to a significant reduction in ulcer index, which indicates the role of reactive oxygen species in drug-induced gastric ulceration. The optimized formulations underwent additional in vivo investigations, which included the evaluation of anti-inflammatory activity by Carrageenan-induced rat paw edema (with paw volume assessed using water plethysmographs), the measurement of analgesic effects through the Tail-Immersion model, and the evaluation of Indomethacin Raft formulation in inflammatory bowel disease (IBD).

MATERIALS AND METHODS

Indomethacin and Quercetinwere received as generous gift samples from Yarrowchem, Mumbai, India. Pectin, Guargum, Mannitol and sodium bicarbonate was obtained fromSDFineChem.Limited,Mumbai. All other reagents used were of analytical grade.

Experimental Animals: Animal experiments were initiated after obtaining the prior approval from IAEC of Sree Siddaganga College of Pharmacy Clear No. Nide Dated

Animals were received from the Animal house of SSCP, Acclimatized for lab conditions for 2 to 5 days, then randomized based on their body weight.

Methodology Experimental Design

The central composite design and response surface methodology were employed to optimize the Raft Forming formulation using Design Expert® software (version 11.1.2.0). The experiment was structured with three levels, two factors, and one process parameter. The key formulation variables—Pectin (mg) (X1), Quercetin (mg) (X2), and Wet Granule Thickness (mm) (X3)—were selected as independent variables, while the dependent variables included Shape & Thickness (mm) (Y1) and In-vitro Dissolution (%) (Y2).

A total of 24 experimental runs were generated based on the levels specified by the Design Expert® software. All formulations prepared according to the experimental design were evaluated for Shape & Thickness (Y1) and In-vitro Dissolution (Y2), which were considered response or dependent variables. The observed responses were analyzed using various mathematical models, including linear, two-factor interaction (2FI), quadratic, and linear models. Statistical significance of the generated models and model terms was assessed using Analysis of Variance (ANOVA). Additionally, 2D contour plots and perturbation graphs generated by Design Expert® software were utilized to examine the relationship between the independent and dependent (response) variables.

Table No.1: Variables used in Central Composite Design

Variables	Low (-1)	Medium (0)	High (+1)
X1: Pectin (mg)	50	75	100
X2: Quercetin (mg)	50	100	150
X3: Granules thickness (mm)	2	3	4

Preparation of Raft Forming Floating Tablet of Indomethacin

Floating raft-forming Indomethacin tablets were formulated using the wet granulation method. The tablet production process involved several steps, including sieving, mixing, lubrication, and compression. Pectin and guar gum were incorporated as viscous gel-forming agents, while sodium bicarbonate served as a gas-generating agent. Mannitol was used

as a diluent, talc as a lubricant, and starch as a binder. The powder blend was compressed into tablets using a rotary tablet punching machine, with each tablet weighing 450 mg, ranging from batch F1 to F24, with varying polymer concentrations as shown in Table no 2 .[9]

Table No. 2: The compositionsof Raft Forming Tablets.

Formulation code	Indomethacin	Quercetin	Pectin	Guar gum	Na2co3	Mannitol	Strach	Talc	Total
F1	50	100	50	50	50	150	20	5	450
F2	50	150	100	50	50	55	20	5	450
F3	50	150	75	50	50	150	20	5	450
F4	50	50	100	50	50	100	20	5	450
F5	50	100	50	50	50	175	20	5	450
F6	50	150	75	50	50	125	20	5	450
F7	50	50	75	50	50	75	20	5	450
F8	50	50	50	50	50	100	20	5	450
F9	50	100	50	50	50	75	20	5	450
F10	50	50	75	50	50	150	20	5	450
F11	50	100	75	50	50	50	20	5	450
F12	50	100	100	50	50	150	20	5	450
F13	50	100	100	50	50	50	20	5	450
F14	50	100	75	50	50	125	20	5	450
F15	50	150	75	50	50	75	20	5	450
F16	50	100	75	50	50	75	20	5	450
F17	50	100	50	50	50	100	20	5	450
F18	50	50	75	50	50	100	20	5	450
F19	50	100	100	50	50	75	20	5	450
F20	50	50	75	50	50	25	20	5	450
F21	50	150	50	50	50	125	20	5	450
F22	50	100	100	50	50	125	20	5	450
F23	50	100	75	50	50	125	20	5	450
F24	50	150	75	50	50	50	20	5	450

Evaluation and Characterization

Before preparing all 24 tablets, the pre-formulation studies were performed [10,11,] such asDrug excipients physical compatibility study by FTIR, AngleofRepose, BulkDensity, Tapped Density, Compressibility Index (Carr's Index) Hauser'sRatio and Post-Compression Parameters [12] such as Shape ofTablets, Hardness, Friability, Weight Variation Test

Floating lag time Studies[13]:

The tablets were placed in a 100 mL beaker containing 0.1 N HCl, and the time taken for the tablet to rise to the surface and begin floating was recorded as the floating lag time.

Raft Strength Measurement [14] :

A tablet powder equivalent to a unit dose was transferred to 150 mL of 0.1 N HCl and maintained at 37°C in a 250 mL glass beaker. Each raft was allowed to form around an L-shaped wire probe (9 cm in height and 2 cm wide at the bottom surface), which remained upright in the beaker throughout the 30-minute raft development period. Raft strength was measured using the modified balance method, where water was added dropwise to the left and right sides of the beaker, and the weight of water required to break the raft was recorded.

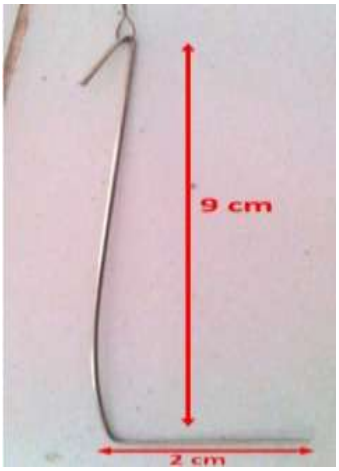

Figure.4 :Wire probe for raft strength measurement

Figure.5:Modified apparatus for raft strength measurement

In-Vitro Drug Release Study:

In vitro drug release studies were conducted to determine the amount of drug released over a specific time period. These studies were performed for all formulations using the USP Type II (paddle method) tablet dissolution apparatus. The dissolution medium consisted of 0.1 N HCl (pH 1.2), maintained at 37 ± 0.5°C, with a paddle rotation speed of 50 rpm. At predetermined time intervals, aliquots were withdrawn, and an equal volume of fresh medium was added to maintain sink conditions. The collected samples were analyzed using UV spectroscopy at 320 nm, with 0.1 N HCl (pH 1.2) as the blank.

Pharmacological Evaluation of anti-inflammatory activity in carrageen an induced paw oedema model[15]

The anti-inflammatory activity was evaluated using a carrageenan-induced paw edema animal model. The experimental animals were randomised into three groups (n=6) based on their body weight.

1. **Group I:** Received carrageenan (1 ml normal saline p.o. + 0.1 ml carrageenan in to a sub-plantar injection)
2. **Group II:** Received Indomethacin Raft formulations (450 mg/kg body weight p.o. +0.1 ml carrageenan in to a sub-plantar injection)
3. **Group III (Standard):** Received Diclofenac Sodium (20 mg/kg body weight, p.o. + 0.1 ml carrageenan in to a sub-plantar injection)

In this model, acute inflammation was induced by a sub-plantar injection of carrageenan 0.1 ml into the hind paw of rats, Group I received only normal saline, Groups II and III received the test drug (Indomethacin Raft formulations) and the Diclofenac Sodium as standard drug respectively + 0.1 ml carrageenan in to a sub-plantar injection 30 min after respective treatment. The volume of the hind paw was measured in ml using the plethysmo meter method at different time intervals i.e., 0, 0.5, 1, 2, 3, 5 & 24 hours post-carrageenan injection to assess Anti- inflammatory activity.

The percentage inhibition of paw edema was calculated by using the following formula;

Percentage of edema inhibition $= \frac{Vc-Vt}{Vc} \times 100$

Vc : Volume of edema in control group

Vt : Volume of edema in treated group

Analgesic activity[16]

The experimental animals were randomised in to 6 groups based on their body weight; the analgesic activity was evaluated using the caudal immersion method. This technique is similar to the tail-flick method, as both involve heat stimuli to induce pain, but they differ in the type of heat source used. While the tail-flick method utilizes a coil, the tail immersion method employs hot water. The rest of the procedure remains the same.

The rats were randomized into 6 groups (n=6).

Group 1 –Received Normal saline serves as control group

Group 2- Received Indomethacin Raft formulations (mg/kg) p.o.

Group 3- Indomethacin Raft formulation with Quercetin (Low dose) is given p.o
Group 4 - Indomethacin Raft formulation with Quercetin - Intermediate doses (Optimized formula) is given p.o,
Group 5- Indomethacin Raft formulation with Quercetin (High dose) is given p.o
Group 6 – Received Diclofenac sodiumserves as standard group
Then response was recorded at 0 hr,0.5hr, 1hr, 2hr,3hr, 4hr, 5hr and 6 hr respectively.
During the experiment, the rats were placed in cages, allowing only one-third of their tails to extend outside. Their tails were then immersed in a hot water bath maintained at **55°C** until the rats withdraw their tails. The time taken to respond to the heat stimulus was recorded as the reaction time. A cut-off time of **180 seconds** was set to prevent injury.

$$\% \text{ Analgesia} = \frac{standard - Control}{Cutoff - Control} \times 100$$

Experimental design for Inflammatory Bowel Disease[17,18,19]
Male Wistar albino rats weighing **200–250 g** were used for the experiment. The animals were randomly divided into four groups(n=6), each containing six rats, and were maintained on a standard diet with water **ad libitum**. The groups were categorized as follows:

- **Group I :**Received Normal saline serves as control group
- **Group II :** Inducer (Indomethacin 40 mg/kg body weight)p.o
- **Group III :** Optimized formula (250 mg/kg body weight)p.o
- **Group IV:**Sulfasalazine (100 mg/kg/day) administered orally along with the raft-forming formulation tablet p.o

Experimental Procedure:
All animals were subjected to a 48-hour fasting period. Group B received no treatment except for ulcer induction with indomethacin (40 mg/kg body weight, orally). Similarly, Groups C and D also received Indomethacin (40 mg/kg body weight, orally) for ulcer induction.
All tablet solutions were freshly prepared 30 minutes prior to administration and given orally. Following Indomethacin administration, the animals were kept without food and water for 9 hours to allow ulcer formation.
Statistical Analysis:
The one-way analysis of variance (ANOVA) was employed for data analysis and results expressed as mean±SD at $p \leq 0.05$ was considered significance.

RESULTS AND DISCUSSION

Pre- formulation studies: Drug-excipients compatibility study

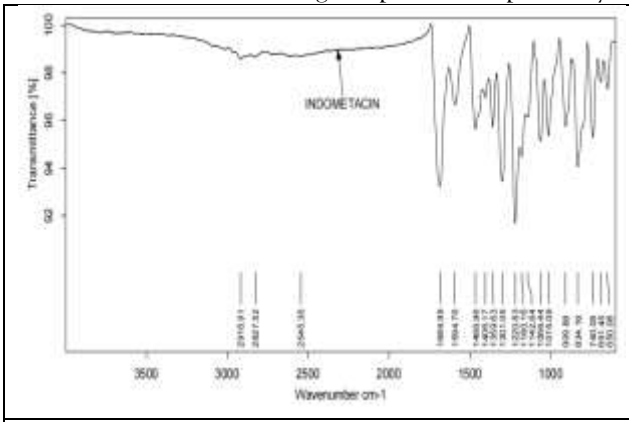

Fig11-FT-IRspectrumofpureIndomethacin.

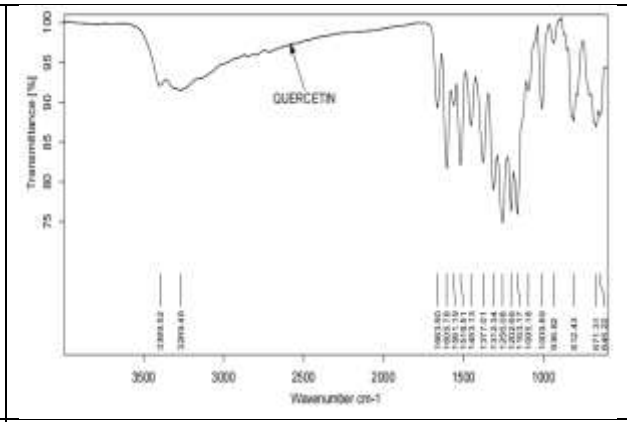

Fig12-FT-IRspectrumofQuercetin

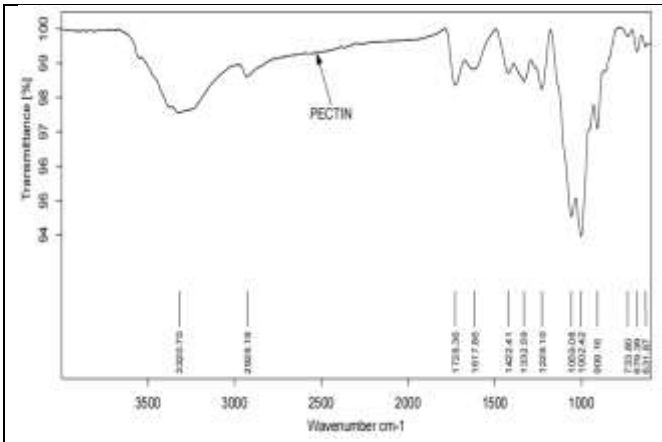

Fig13-FT-IRspectrumPectin

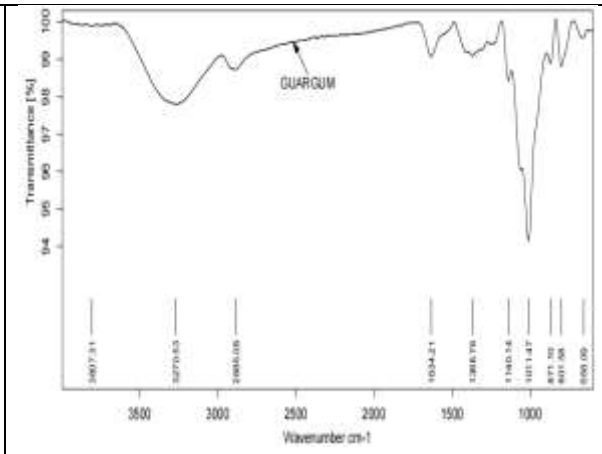

Fig14-FT-IRspectrumGuargum

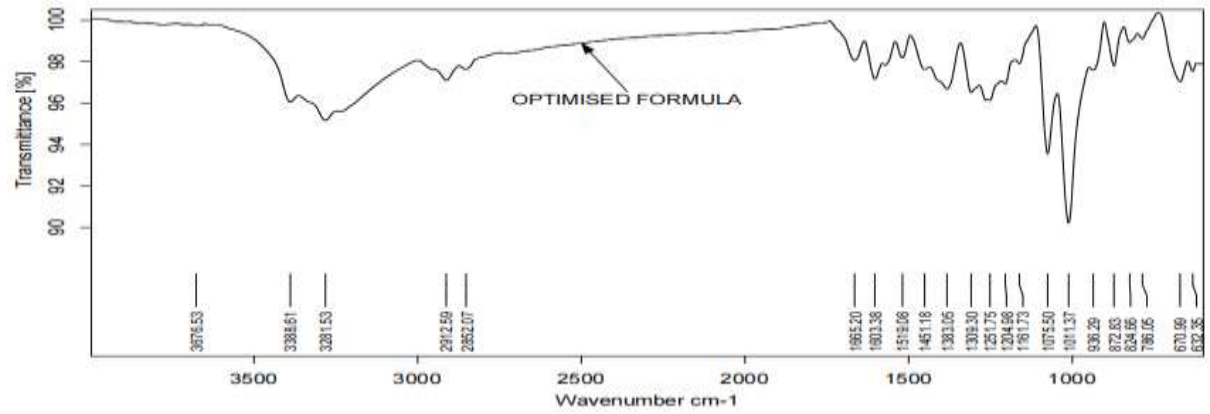

Fig15-FT-IRspectrumofoptimizedformulation

Table No: 03 Pre-compression evaluation data

Formulation Code	Bulk density (gm/cm^3)	Tap density (gm/cm^3)	Carr's index (%)	Hausner's ratio	Angleof repose (θ)
F1	0.38±0.03	0.41±0.08	6.44±0.03	1.07±0.03	24.81±0.05
F2	0.39±0.07	0.40±0.30	3.34±0.16	1.02±0.05	25.39±0.75
F3	0.39±0.11	0.42±0.14	6.64±0.27	1.06±0.08	28.65±0.02
F4	0.37±0.21	0.39±0.31	6.27±0.32	1.05±0.09	24.81±0.25
F5	0.41±0.04	0.39±0.31	4.65±0.12	1.04±1.08	27.74±0.13
F6	0.39±0.41	0.42±0.08	3.34±0.27	1.07±0.31	28.65±0.82
F7	0.39±1.09	0.40±0.15	3.36±0.21	1.02±0.21	25.48±1.15
F8	0.37±0.34	0.39±0.31	5.45±0.27	1.05±0.11	27.55±0.87
F9	0.41±0.51	0.43±0.02	4.65±0.16	1.04±0.98	25.81±0.06
F10	0.42±0.01	0.45±0.07	10.66±0.14	1.07±0.08	31.81±0.03
F11*	**0.39±0.07**	**0.40±0.30**	**3.34±0.16**	**1.02±0.05**	**25.39±0.75**
F12	0.42±0.01	0.45±0.07	10.66±0.14	1.07±0.08	30.81±0.03
F13	0.46±0.05	0.48±0.06	4.16±0.23	1.14±0.01	28.39±1.05

F14	0.42±0.11	0.45±0.35	9.69±0.19	1.07±0.05	30.81±0.22
F15	0.42±0.11	0.55±0.35	9.09±0.19	1.04±0.05	31.91±0.02
F16	0.32±0.07	0.46±6.12	7.14±0.19	1.09±0.09	30.21±0.82
F17	0.37±0.34	0.39±0.31	5.45±0.27	1.05±0.11	27.55±0.87
F18	0.37±0.21	0.39±0.31	6.27±0.32	1.05±0.09	24.81±0.25
F19	0.39±1.09	0.40±0.15	3.36±0.21	1.02±0.21	25.48±1.15
F20	0.39±0.15	0.42±0.36	3.15±0.15	1.07±0.02	30.76±0.03
F21	0.42±0.11	0.45±0.35	9.69±0.19	1.07±0.05	30.81±0.22
F22	0.41±0.01	0.45±0.04	8.78±0.27	1.09±0.13	29.93±0.11
F23	0.40±0.12	0.43±0.11	6.97±0.18	1.06±0.03	28.55±0.02
F24	0.42±0.04	0.45±0.15	6.66±0.16	1.07±0.07	30.81±0.15

Formulation code	**Hardness (Kg/cm2)**	**Thickness (mm)**	**Friability (%)**	**weight variation(gm)**	**Drug content**
F1	3.21±0.2	4.01±0.12	0.45±0.08	446.34±0.24	97.10±2.07
F2	3.95±0.5	3.1±0.32	0.38±0.02	440.51±0.33	98.20±1.95
F3	3.01±0.9	2.03±0.25	0.32±0.03	459.83±0.36	95.50±063
F4	3.81±1.3	4.00 ±0.12	0.40±0.09	450.21±0.49	95.83±3.03
F5	3.02±0.2	3.18±0.22	0.45±0.02	450.72±0.30	98.65±0.25
F6	4.51±0.5	2.21±0.19	0.30±0.01	439.64±0.11	97.40±1.30
F7	2.63±0.9	3.0±0.21	0.25±0.09	435.68±1.32	95.94±1.74
F8	3.08±0.1	2.01±0.18	0.42±0.01	441.96±0.24	98.30±1.07
F9	3.50 ±1.2	3.04±0.29	0.45±0.02	450.71±0.20	98.10±0.15
F10	4.10 ±1.2	3.01±0.15	0.49±0.01	465.82±0.18	99.63±0.49
F11*	**3.95±0.5**	**3.1±0.32**	**0.38±0.02**	**440.51±0.33**	**98.20±1.95**
F12	4.10 ±1.2	3.01±0.15	0.49±0.01	465.82±0.18	99.63±0.49
F13	3.81±1.0	4.15±0.35	0.33±0.73	449.51±0.68	97.30±0.83
F14	5.98±0.9	3.09±0.41	0.38±0.05	460.61±0.07	97.41±1.09
F15	3.03±0.12	4.02±0.15	0.25±0.03	439.82±0.61	98.01±3.01
F16	4.97±0.5	2.09±0.29	0.33±0.07	450.86±0.09	97.61±1.88
F17	3.08±0.1	2.01±0.18	0.42±0.01	441.96±0.24	98.30±1.07
F18	3.81±1.3	4.0±0.12	0.45±0.09	450.21±0.49	95.83±3.03
F19	2.63±0.9	3.0±0.21	0.25±0.09	435.68±1.32	95.94±1.74
F20	4.96±0.2	3.19±0.43	0.45±0.07	447.72±0.81	97.04±2.62

F21	5.98±0.9	3.09±0.41	0.38±0.05	460.61±0.07	97.41±1.09
F22	2.03±1.3	4.02±0.11	0.45±0.01	450.70±0.59	99.14±0.59
F23	4.31±0.8	3.0±0.13	0.21±0.08	470.53±0.48	95.11±4.99
F24	3.80 ±1.9	2.13±0.73	0.45±0.03	451.63±0.19	98.30±0.92

Table No.04: Post Compression Parameters
Each value represents the mean ± standard deviation (n=3)

Table No.05: Post CompressionParameters.

Formulation code	**Floating lag time (sec)**	**Total floating time (hour)**	**Raft Strength (g)**	***In-vitro* drug release**
F1	45	10	3.09±0.15	93.14±2.07
F2	55	10	7.09±0.18	73.15±1.05
F3	42	09	5.07±0.83	85.89±1.59
F4	59	10	7.91±0.04	70.69±1.07
F5	45	10	3.25±0.07	93.86±1.83
F6	50	10	5.38±0.12	86.91±1.25
F7	51	10	5.15±0.02	88.01±2.08
F8	45	10	2.06 ±0.03	90.31±0.63
F9	43	10	6.18±0.15	90.01±3.50
F10	51	10	5.15±0.02	88.01±2.08
F11*	**49**	**10**	**4.96±0.93**	**89.72±1.03**
F12	58	10	7.83±0.29	75.81±2.30
F13	59	10	8.29±0.34	72.71±1.82
F14	49	10	4.96±0.93	84.72±1.03
F15	50	10	5.38±0.12	86.91±1.25
F16	53	09	6.10±0.13	80.72±4.27
F17	45	10	3.25±0.07	93.86±1.80
F18	52	10	4.85±0.06	88.82±1.73
F19	60	09	7.83±0.29	75.81±2.30
F20	52	10	5.89±0.10	87.93±2.87

F21	40	10	3.68±0.26	97.50±1.31
F22	58	10	6.90 ±0.92	76.81±2.52
F23	53	10	6.10±0.13	80.72±4.27
F24	48	09	4.78±0.35	85.92±0.93

Each value represents the mean ± standard deviation (n=3)

Table No.06: Experimentalresults

Experimentalrun	**Pectin (mg)**	**Quercetin (mg)**	**Granules thickness (mm)**	**Response (Y1) Shape& thickness (mm)**	**Response (Y1) In-vitro dissolution (%)**
F1	50	100	2	4.01±0.12	93.14±2.07
F2	100	150	3	3.1±0.32	73.15±1.05
F3	75	150	2	2.03±0.25	85.89±1.59
F4	100	50	3	4.00 ±0.12	70.69±1.07
F5	50	100	3	3.18±0.22	93.86±1.83
F6	75	150	3	2.21±0.19	86.91±1.25
F7	75	50	3	3.0±0.21	88.01±2.08
F8	50	50	3	2.01±0.18	90.31±0.63
F9	50	100	4	3.04±0.29	90.01±3.50
F10	75	50	3	3.01±0.15	88.01±2.08
F11*	75	100	3	3.1±0.32	89.72±1.03
F12	100	100	3	3.01±0.15	75.81±2.30
F13	100	100	2	4.15±0.35	72.71±1.82
F14	75	100	4	3.09±0.41	84.72±1.03
F15	75	150	3	4.02±0.15	86.91±1.25
F16	75	100	2	2.09±0.29	80.72±4.27
F17	50	100	3	2.01±0.18	93.86±1.83
F18	75	50	2	4.0±0.12	88.82±1.73
F19	100	100	3	3.0±0.21	75.81±2.30
F20	75	50	2	3.19±0.43	87.93±2.87
F21	50	150	3	3.09±0.41	97.50±1.31

F22	100	100	4	4.02±0.11	76.81±2.52
F23	75	100	2	3.0±0.13	80.72±4.27
F24	75	150	2	2.13±0.73	85.92±0.93

***In-vivo* studies**

Table No. 07: Anti-inflammatory activity using carrageenan induced paw oedema model(reference)

Groups	Treatment	Mean increase in aw edema ± SEM						
		0 hr	0.5 hr	1 hr	2 hr	3 hr	5 hr	24 hr
1	Inducer	1.563± 0.00088	34.36± 1.667****	44.86± 0.0016****	56.13± 0.00033****	42.21± 0.0033****	58.31± 0.59****	45.64± 0.035****
2	RFF IND	1.539± 0.00088	4.688± 0.033****	9.721± 0.00057****	19.96± 0.0033****	25.03± 0.0033****	28.03± 0.14****	28.18± 0.030****
3	STD	1.540± 0.00088	2.757± 0.066****	9.340± 0.0057****	16.05± 0.0033****	20.42± 0.0033****	25.05± 0.28****	25.63± 0.028****

Each Value are expressed as Mean ± SEM (n=6) by one way ANOVA followed by Tukey test. Where,
* represent significant at p<0.05, **(p<0.01), ***(p<0.001), **** (p<0.0001).

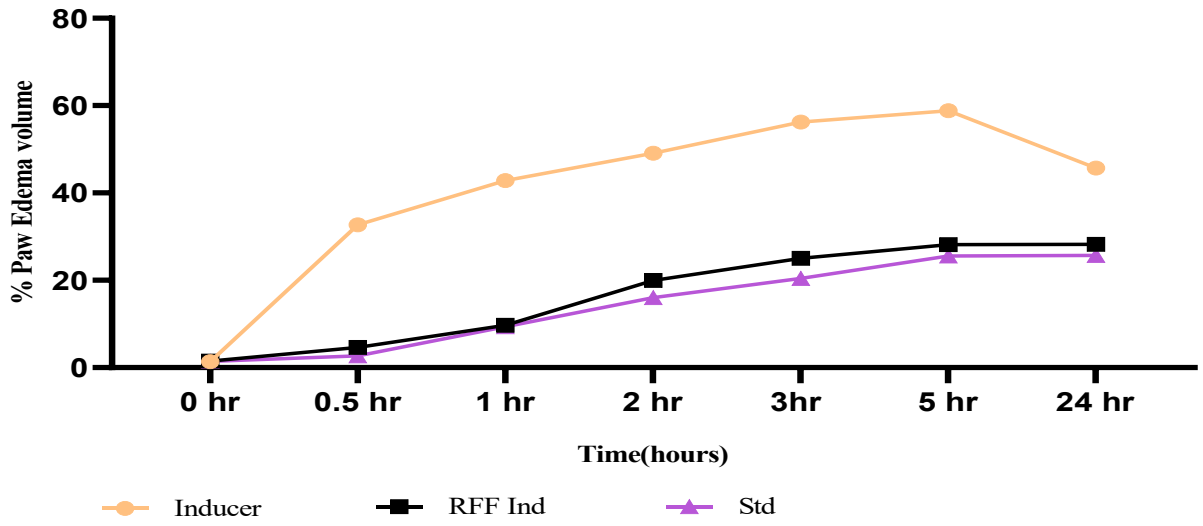

Figure 16: Anti-inflammatory effect by carrageenan induced paw edema model

Change in paw thickness (cm) at t = 0, 0.5, 1, 2, 3, 5, and 24 hours. n = 6 (significant at P < 0.001). Edema was induced by injecting 0.1mL of 1% solution of carrageenan into the sub plantar
surface of right-hind paw. Data are expressed as mean ± standard error of 6 rats per group. Group 1: Carrageenan control; Group 2: RFF Ind; Group 3: Standard;

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Analgesic activity by Tail immersion method

Table No. 08: Reaction time in seconds at different time interval

Groups	Treatment	Mean increase in paw edema ± SEM							
		0 hr	0.5 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
G1	Control	2.904± 0.00033	2.822± 0.0003	3.098± 0.0033	3.468± 0.00033	2.572± 0.0033	1.832± 0.00033	1.410± 0.00033	1.170± 0.00033
G2	RFF IND	2.877± 0.00033 ns	9.460± 0.0003 ****	15.65± 0.0033 ****	18.24± 0.0033* ***	18.73± 0.0057*** *	3.652± 0.00088 ****	2.315± 0.00033 ****	1.878± 0.00033* ***
G3	T1(low dose)	3.365± 0.00033 ****	7.852± 0.0003 ****	16.65± 0.0033 ****	23.53± 0.00033 ****	24.06± 0.0057*** *	17.92± 0.00088 ****	3.112± 0.00033 ****	1.723± 0.00033* ***
G4	T2(medium dose)	3.687± 0.00033 ****	6.798± 0.0057 ****	12.43± 0.0033 ****	19.07± 0.0033* ***	24.34± 0.0033** **	18.34± 0.040*** *	3.757± 0.00033 ****	2.489± 0.00066* ***
G5	T3(high dose)	3.553± 0.00033 ****	10.23± 0.0057 ****	26.56± 0.0033 ****	28.47± 0.00033 ****	27.71± 0.00033* ***	14.30± 0.010*** *	2.865± 0.00033 ****	2.567± 0.00033* ***
G6	Std (Diclofenac)	3.193± 0.00033 ****	20.30± 0.0057 ****	26.44± 0.0066 ****	28.71± 0.00033 ****	6.903± 0.00033* ***	3.512± 0.00033 ****	3.121± 0.00066 ****	2.143± 0.00033* ***

Values are expressed as Mean ± SEM (n-6) by one way ANOVA test. Where, * represent significant at p<0.05,**(p<0.01), ***(p<0.001), **** (p<0.0001) was considered as significant when compared to control.

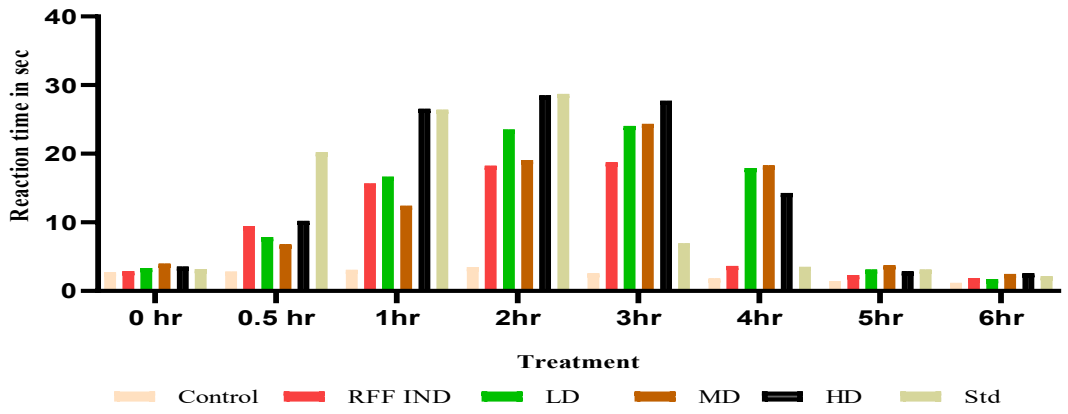

Fig no. 17: Plot ofAnalgesicEffect of Tail -Immersion method

Inflammatory Bowel Disease Activity

Table No. 09: Effect of Raft forming Indomethacin Tablet on LDH, GSH, LPO, CAT (catalase Indomethacin induced)

SI NO	Groups	LDH(U/L)	GSH(µM/g)	LPO(µM/g)	CAT(U/g)

1	Normal Control	456.8±60.25****	3.382±0.2548****	1.919±0.3173 ****	3.735±0.4860****
2	Inducer pure drug Indomethacin (40 mg/kg, p.o.)	1553±157.3 ####	0.5692±0.1444 ####	12.48±2.343 ####	0.501±0.14####
3	Optimized formula (250mg/kg, p.o.)	604.5±86.14 ns	2.179±0.1913**	2.093±0.4527 ****	2.491±0.37**
4	Standard Sulphasalazin (250mg/kg, p.o.)	700.8±95.16 ns	2.328±0.4006***	2.207±0.31****	2.360±0.46**

Values are expressed as Mean ± SEM (n-6) by one way ANOVA followed by Bartlett's test. * represent significant at p<0.001,** (p<0.01), *** (p<0.001), **** (p<0.0001) was considered as compared to control.

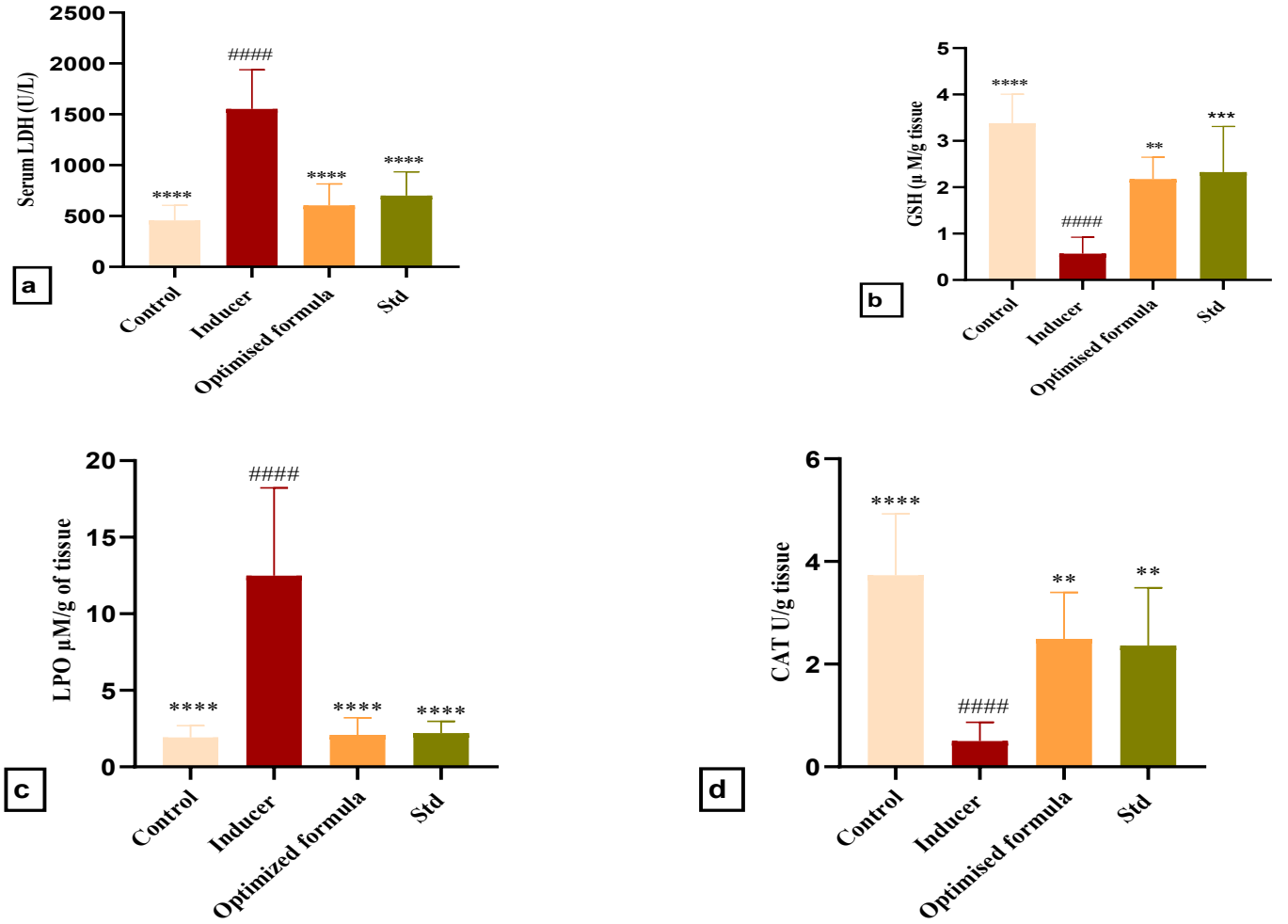

Fig no. 18: Effect of Raft forming Indomethacin Tablet on [a]-LDH, [b]-GSH, [c]-LPO, [d]- CAT using in different groups in Indomethacin induced model.

Table No. 10: Raft forming Indomethacin Tablet on Pathological changes in rat stomach induced by inducer

Groups	Treatment	Ulceration	Hyperemia	Necrosis	Edema	Cellular infiltration
1	Normal Control	Nil	Nil	Nil	Nil	Nil

2	Inducer Indomethacin (40mg/kg,p.o.)	***	**	**	***	***
3	Optimized formal (250mg/kg, p.o.)	Nil	*	Nil	*	Nil
4	Standard Sulphasalazin (250mg/kg, po)	Nil	Nil	Nil	*	Nil

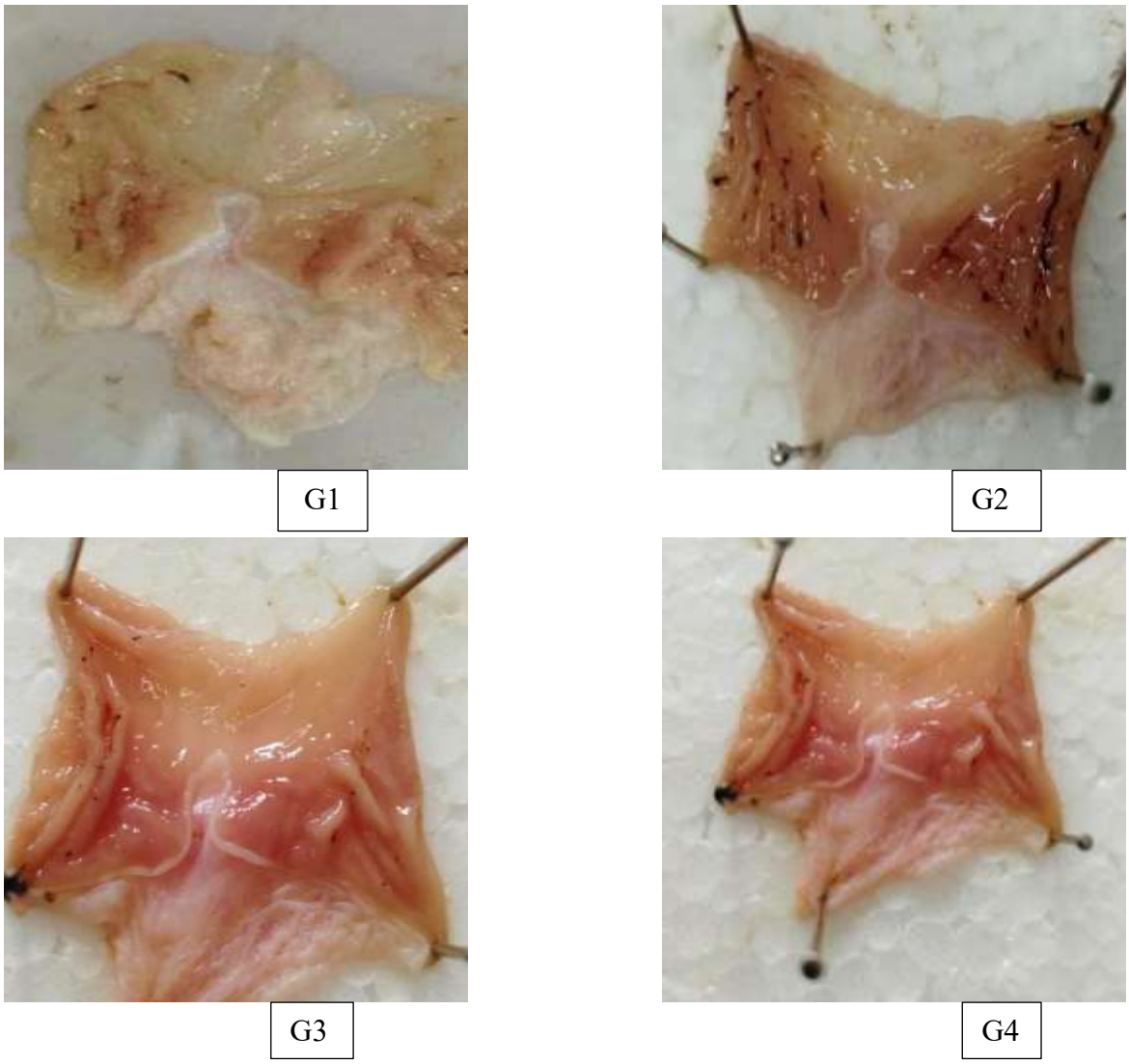

Fig no. 19: Effect of pre- treatment of RFF IND on Stomach morphology. [G1] Normal Control; [G2] Inducer Indomethacin (40mg/kg,p.o.); [G3]Optimized formal (RFF IND)(250mg/kg,p.o.);[G4] Sulphasalazin(100mg/kg,p.o.)

Table No. 11: Effect of Raft forming Indomethacin Tablet on Pathological changes in rat Ileum induced by Inducer.

Groups	Treatment	Ulceration	Hyperemia	Necrosis	Edema	Cellular Infiltration
1	Normal Control	Nil	Nil	Nil	Nil	Nil

2	Inducer Indomethacin (40mg/kg,p.o.)	***	***	***	****	***
3	Optimized formal (250mg/kg, p.o.)	Nil	*	Nil	**	Nil
4	Std Sulphasalazin (250mg/kg, po)	Nil	*	Nil	**	Nil

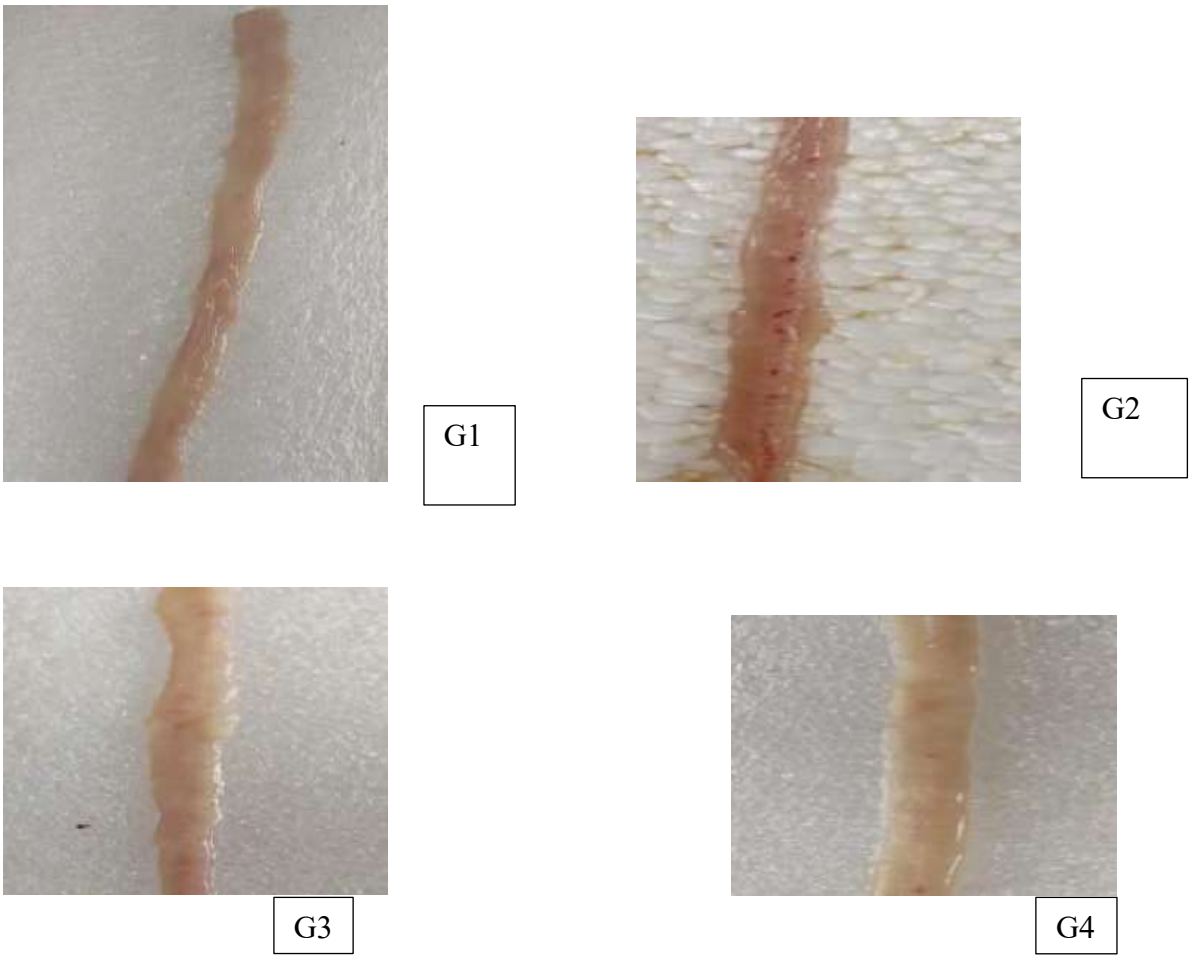

Fig no. 20: Effect of pre- treatment of RFF IND on Ileum morphology. [G1] Normal Control; [G2] Inducer Indomethacin (40mg/kg,p.o.); [G3] Optimized formal (RFF IND) (250mg/kg,p.o.) ;[G4] Sulphasalazin (100mg/kg,p.o.)

DISCUSSION

The FTIR spectral analysis was conducted to determine the compatibility between Indomethacin and the polymers used in the raft system formulation. The optimized formulations exhibit the characteristic functional groups of Indomethacin, with peaks at 1603–1519 cm^{-1} and 1451 cm^{-1} corresponding to (C-C) stretching of the aromatic rings, and a peak at 1665 cm^{-1} indicating the presence of amide groups. A strong OH stretching band at 3388 cm^{-1} and 3281 cm^{-1}, attributed

to hydroxy group stretching, confirms the presence of Quercetin. The peak at 2912 cm^{-1} corresponds to C-H stretching, while the peak at 1011 cm^{-1}, indicative of secondary alcohol, confirms the presence of Pectin. Additionally, the peak at 2852 cm^{-1} signifies the presence of -NH groups, whereas the peak at 1383 cm^{-1}, representing C-O-C stretching vibrations of the glycosidic linkage, indicates the presence of Guar gum. The presence of functional groups from both the drug and polymers in the optimized formulation confirms that there is no interaction between the ingredients used in the preparation of the raft-forming formulations.The angle of repose for all 24 formulations ranged from 24°.81' to 31°.91', indicating good powder flow properties, as all values were below 30°. The loose bulk density and tapped bulk density varied between 0.32–0.43 gm/cm^3 and 0.39–0.55 gm/cm^3, respectively. These values fall within acceptable limits, with minimal differences between loose and tapped densities, aiding in the calculation of powder compressibility.The percentage compressibility ranged from 3.44% to 10.66%, signifying good to excellent flow characteristics of the tablet mixture. Hausner's ratio for all formulations was between 1.02 and 1.09, further confirming excellent powder flow. Pre-compression parameters, including the angle of repose, bulk density, tapped bulk density, percentage compressibility, and Hausner's ratio, were all within acceptable limits, as shown in Table 3.The hardness of all 24 formulations was maintained between 2.6 and 4.9 kg/cm^2, which is crucial for the tablet's overall performance and efficacy, with the standard hardness being 4 kg/cm^2. Tablet thickness ranged from 2.01±0.18 mm to 4.15±0.35 mm. The friability was controlled between 0.21±0.08% and 0.45±0.08%, remaining well below the standard limit of 1%. All formulations passed the weight variation test, with weight variation within the pharmacopeial limit of 5% of the average weight. The drug content across all formulations ranged from 95.11% to 99.63%. The hardness, thickness, friability, weight variation, and drug content values, as detailed in Table 4, were all within specified limits.

In the in-vitro buoyancy studies, tablets from formulations F1 to F24 were immersed in 0.1N HCl solution at 37±5°C. The tablets floated and remained buoyant without disintegration. The buoyancy lag time (BLT) ranged from 42 to 59 seconds, while the total floating time (TFT) varied between 9 to 10 hours, as presented in Table 5.The in-vitro raft strength of formulations F1 to F24 was measured using an in-house method, with values ranging from 3.09±0.15 to 8.29±0.34 gm, also detailed in Table 5.

Design of experiments methodology has been done by initial input parameters,such as formulation components, process variables, and their respective levels, are first entered into the software. These inputs provide the framework for the software to design a set of experimental runs that systematically vary the factors of interest. The software then analyses the experimental data to determine the relationships between the variables and the desired outcomes. Statistical methods are applied to identify significant factors and interactions affecting the formulation's performance. Based on this analysis, the software generates an optimized formulation Anova analysis have been conducted for response variable,as per the Central Composite Design (CCD), formulated 24 batches were assessed, to evaluate the impact of independent variables (coded as A and B) on the responses: Shape & Thickness (R1) and In-vitro Release Studies (R2). The results for these batches are presented in Table 6. The software compares the experimental and predicted values for model evaluation, after complete analysis, it generates optimal formula of the factors with desired responses to give optimized formulation (Formulation 11).

Response 1: Shape and Thickness (R1): The lowest Shape and Thickness (2.01 mm) was recorded in Run 8, whereas the highest (4.15 mm) was observed in Run 13. The influence of independent variables and their interactions were analysed using ANOVA, which yielded an F-value of 939.32, a p-value of 0.0050, and an adjusted R^2 of 0.9916. These results indicate that the model is statistically significant and follows a Quadratic model.The ANOVA findings confirm that the response has been significantly affected by the independent variables.

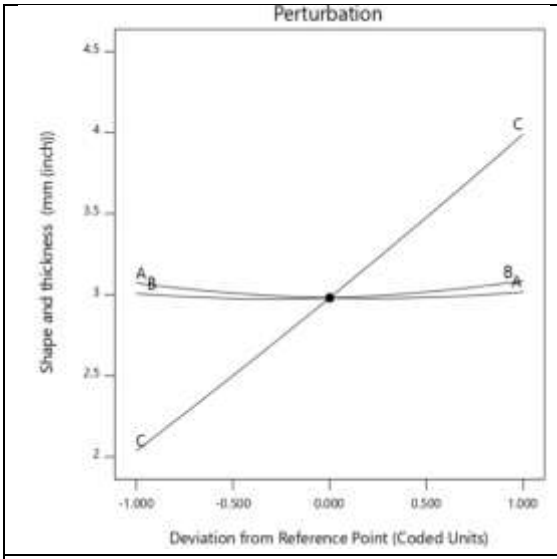

Fig.20 - Perturbation of Shape& thickness (mm)

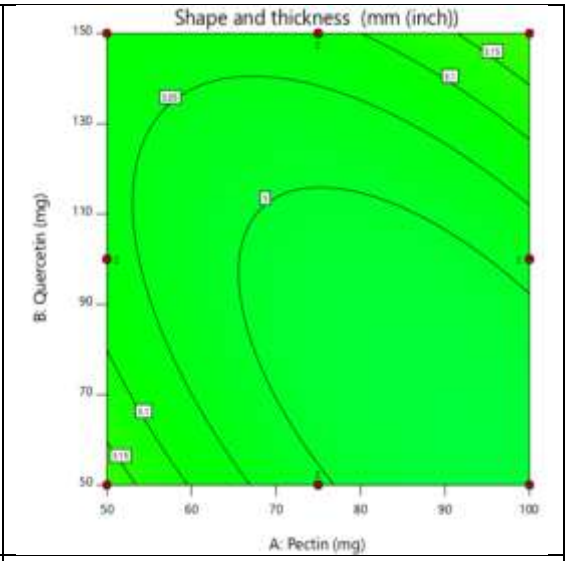

Fig.21 - 2D graph of Shape & thickness (mm)

Response2:*In-vitro* dissolution (%) R2:The lowest drug release (R2) was observed in Run 4 (70.69%), while the highest (99.50%) was recorded in Run 21. The significance and interaction of the independent variables were analysed using ANOVA, which produced an F-value of 45.53, a p-value of 0.0001, and an adjusted R^2 of 0.8531. These results confirm that the model is statistically significant and follows a Linear model. The ANOVA results indicate that the independent variables significantly influenced the response. The polynomial equation for Response 2 (R2) is expressed as:

Y(R2) =84.62 −9.45A + 0.2700+0.5200 ⋯⋯⋯⋯ **(2)**

In the polynomial equation, positive coefficients represent a synergistic effect, meaning the factors enhance the response. Conversely, negative coefficients indicate an antagonistic effect, meaning the factors reduce the response.To provide a more detailed interpretation of the design, 3D response surface plots were generated to illustrate the interaction of the three factors (A, B, and C) on Response 2 (R2), as depicted in Figure 22.

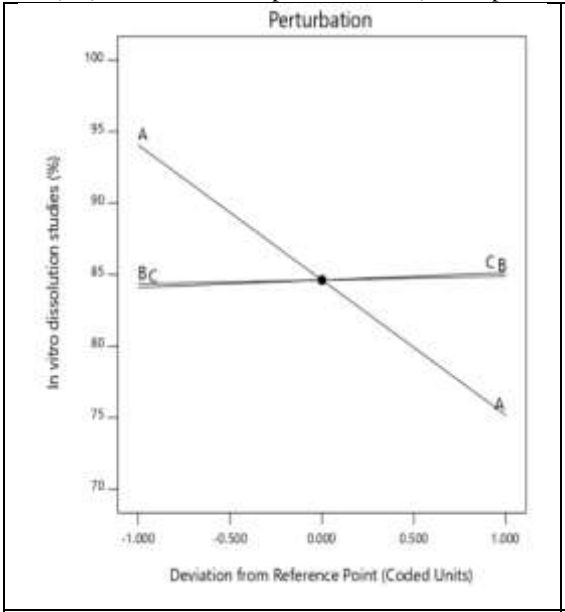

Fig.22 - Perturbation of *In-vitro* dissolution

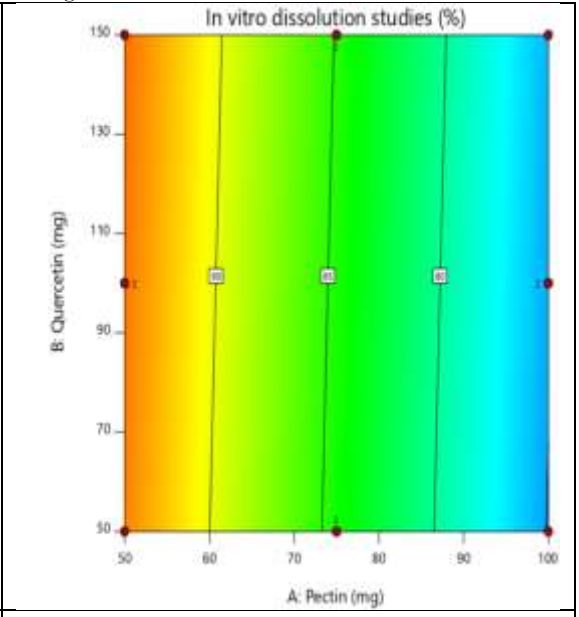

Fig.23 - 2D graph of In-vitro dissolution

"The carrageenan-induced rat paw edema model is a well-established method for evaluating anti-inflammatory drugs and is frequently used to assess their antiedematous effects". "Carrageenan is a potent phlogistic agent used to induce the

release of inflammatory and proinflammatory mediators such as prostaglandins, leukotrienes, histamine, bradykinin, and TNF-α."[20]The course of acute inflammation is biphasic. The initial phase occurs within the first few hours following the administration of a phlogistic agent and is characterized by the rapid release of vasoactive mediators such as histamine, serotonin, and kinins.[21]The second phase, occurring within 2–3 hours, is associated with the release of prostaglandin-like substances and is sensitive to both steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs).[22]

Although both cyclooxygenase and lipoxygenase pathways contribute to inflammation, cyclooxygenase inhibition is more effective in suppressing carrageenan-induced inflammation than lipoxygenase inhibition.[23]Sub plantar administration/Injection of carrageenan into the hind paw induced progressively increasing edema, peaking at 4 hours. At baseline (t = 0 hours), there were no significant differences in paw volume among the experimental groups and induction group. The mean paw volumes were as follows: Inducer group – 1.563 ± 0.00088, RFF IND– 1.539 ± 0.00088, and Standard control – 1.540 ± 0.00088. However, a marked and statistically significant increase in paw volume was observed in the Inducer group ($P < 0.0001$), indicating successful induction of inflammation. In comparison, all treatment groups demonstrated a significant reduction in paw volume relative to the Inducer group at different time intervals and values were represented in the above **Table No. 7**and **Figure No.16** indicating a sustained anti-inflammatory effect of the test compounds.

The analgesic potential of the optimized RFF IND formulation was assessed using the tail immersion method. The study included six groups: control (G1), RFF IND (G2), low dose (G3), medium dose (G4),high dose(G5), and a standard drug group (G6). No significant change in reaction time was observed in the control group (G1) throughout the observation period. At the 0-hour, baseline reaction time for groups G2, G3, G4, and G5 were recorded in **Table No. 8** and **Figure No.17**All treatment groups showed a significant increase in reaction time ($p < 0.0001$), reaching a maximum at 3 hours of post-administration of G2, G3, G4, G5.

This indicates a time- and dose-dependent increase in analgesic activity, with peak effects observed at 3 hours. After this peak, reaction time declined with respect to increase in time across all groups.

The standard group (G6) and other Groups (G2, G3, G4 and G5) exhibited the highest analgesic response in comparison to the control group. These results suggest that the optimized RFF IND formulation demonstrates significant analgesic activity, particularly at higher doses, and maintains effectiveness comparable to the standard drug,

All experimental animal groups were subjected to Inflammatory Bowel Disease (IBD) induction and assessed for various inflammatory and oxidative stress markers, including Lactate Dehydrogenase (LDH), Glutathione (GSH), Lipid Peroxidation (LPO), and Catalase (CAT). This study evaluated the effects of an Optimized Formula (250 mg/kg), with Indomethacin (40 mg/kg) used as the disease inducer, and Sulfasalazine (100 mg/kg) as the standard reference drug, in combination with a raft-forming formulation.

The results demonstrated that the inducer group exhibited a significant increase in LDH levels compared to the normal control group. In contrast, both the treatment groups (G3) and the standard group showed a marked reduction in LDH levels. The GSH levels were significantly reduced in the inducer group relative to the normal group, whereas the treatment and standard groups exhibited a restoration and elevation of GSH levels. Regarding LPO levels, there was a notable increase in the inducer group compared to the normal group; however, both the treatment and standard groups showed a significant decrease in LPO concentration. Similarly, CAT activity was diminished in the inducer group compared to the normal control, while the treatment and standard groups displayed enhanced catalase activity, as presented in **Table No. 9**and **Figure No.18** Indicating a protective antioxidant effect.

Macroscopic observation of stomach: In normal control group morphological damage found to be no change with regular mucosal secretion. In Indomethacin induced group shows significant increase in gastric ulceration, hyperaemia and edema in comparison with normal group. In standard exhibited significant protective effect in morphological damage where as group treated with optimized formula also exhibited significant protective effect in morphological damage in comparison with inducer group and results are presented in **Table No. 10** and **Figure No. 19**. Suggesting that optimized formulation may possess gastroprotective activity.

Macroscopic observation of rat ileum: In inducer group significant pathological changes were observed in ileum compared to the normal group in contrast treatment group demonstrated significant protective effect against disruption in mucosal changes and visible signs of inflammation in comparison with inducer group the findings are represented in **Table No. 11** and **Figure No. 20**.

CONCLUSION

In this research, the gastro retentive raft forming Indomethacin tablets were prepared successfully using Quercetin and raft forming polymers to extend the release the Indomethacin without damaging the GIT, as it is a NSAIDs. Quercetin is a polyphenol which having the property of antiulcerogenic, which have been investigated by inflammatory bowel disorder method. Indomethacin Raft forming Tablets were prepared by wet granulation method. Design of experiments methodology has been done by to optimize the formulations. Pre-compression parameters AngleofRepose, BulkDensity, Tapped Density, Compressibility Index (Carr's Index) Hauser'sRatio demonstrated good flow properties within the passable limit, Drug Excipients Physical Compatibility Study (FTIR) confirms all used ingredients are compatible with each other, Post- Compression Parameters such as Shape ofTablets, Hardness, Friability, Weight Variation Test, drug content was reported to be uniform and within the acceptable limit.In-vitro drug release study was performed, all formulations exhibit good drug release up to 10 hours.

The optimized RFF Indomethacin formulations were systematically evaluated for their pharmacological efficacy using established in vivo models. The carrageenan-induced paw edema model demonstrated significant anti-inflammatory activity, while the tail-immersion method confirmed analgesic effects. Additionally, the Indomethacin-induced inflammatory bowel disease model and macroscopic observations of rat stomach and ileum morphological changes further supported the potential of these formulations in managing inflammation-associated gastrointestinal disorders. Collectively, the results suggest that the optimized RFF Indomethacin formulations exhibit promising anti-inflammatory and analgesic properties, supporting their potential therapeutic application in inflammatory and pain-related conditions.

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