Research Article

Fabrication, characterization and *in vivo* evaluation of luteolin loaded microspheres for inflammatory bowel disease

Anil Kumar Pandey, Alok Pal Jain, Anjana Bhardwaj*

RKDF College of Pharmacy, SRK University, Hoshangabad Road, Misrod, Bhopal - 462026 (MP) India

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Abstract

Background: Ulcerations and inflammation occur in ulcerative colitis in the inner lining of the colon, resulting in symptoms of abdominal pain, diarrhoea and rectal bleeding. **Objective:** Aim of present study was to prepare and characterized luteolin loaded microspheres for effective treatment of ulcerative colitis. **Materials and methods:** Pre-formulation studies of luteolin were performed to characterize the drug and then Luteolin microsphere was prepared by solvent evaporation method through different optimized parameters. For characterization of prepared microspheres various parameters like particle size, drug entrapment, percentage yield, shape and surface properties were analysed. Scanning electron microscopy of optimized formulation F1 was confirmed the spherical shape of microspheres. The *in vitro* stability of luteolin microspheres were stored at $5\pm3^{\circ}\text{C}$, $25\pm2^{\circ}\text{C}/60\pm5\%$ RH and $40\pm2^{\circ}\text{C}/75\pm5\%$ RH. Changes in particle size after time interval of 30, 60 and 90 days were determined. DSS produces severe macroscopic edematous inflammation in the colon. The disease activity index and wet colon weight for different groups were observed in DSS induced ulcerative colitis. **Results and conclusion:** The disease activity index and weight of colon for colitis control group were found as 4.12 ± 0.82 , 201.72 ± 4.37 , respectively. The disease activity index and colon weight for LUT and LUT microsphere treated groups of animals were observed significantly decreased as 2.63 ± 0.27 and 1.53 ± 0.62 . LUT Microsphere decreases weight of colon significantly as 133.96 ± 3.21 . In conclusion, the free radical scavenging property of Luteolin plays a significant role in ulcer healing. Significant increase in GSH level and reduction in MDA level has also been revealed in extracts treated groups while investigating *in vivo* antioxidant activity.

Keywords: Luteolin, ulcerative colitis, antioxidant, inflammation, microsphere, dextran sodium sulfate

Introduction

Microparticles are particulate dispersions or solid particles of size range of 1-1000 μm . Depending upon whether drug has been entrapped or dispersed, they have been classified into microcapsules or microspheres. Microcapsules contains well defined core in which drug is enclosed by unique polymer film. Microspheres are matrix system in which drug is physically and uniformly dispersed (Kumar, 2000). Inflammatory bowel disease (IBD), which comprises Crohn disease and ulcerative colitis, characterizes a group of chronic diseases illustrated GIT. The chief reason of IBD are not well implicit, but inequities in

proinflammatory cytokines like TNF- α , IFN- γ , IL-1, IL-6, and IL-12 and anti-inflammatory cytokines as well as IL-4, IL-10, and IL-11 are consideration to participate an innermost character in mediating and modulating inflammation (Jump and Levine, 2004).

Ulcerative colitis (UC) is a rectal and colonic mucosal chronic, idiopathic, inflammatory bowel disease (IBD). It is characterized by colonic inflammation, most likely due to the infiltration of polymorphonuclear cells, lymphocytes, monocytes and plasma cells, accompanied by oxygen-free radicals, which ultimately leads to mucosal alteration and ulceration (Cho et al., 2007).

The oral route is considered as to be the most convenient rout for administration of drug to the patient. Oral administration of conventional dosage forms normally dissolves the drug in the stomach fluid or intestinal fluid and absorb from these regions of GIT depends upon the

*Address for Corresponding Author:

Prof. Anjana Bhardwaj
RKDF College of Pharmacy, SRK University, Hoshangabad road,
Misrod, Bhopal - 462026 (MP) India
Email: anjanab2210@gmail.com

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physiochemical properties of the drug. It is a serious drawback in conditions where localized drugs delivery in the colon is required or in conditions where a drug needs to be protected from the upper GIT hostile environment. Dosage forms that deliver drugs into colon rather than upper GIT offers number of advantages (Tripathi and Feuerstein, 2019). Targeted delivery of drugs to the colon is valuable in the treatment of disease of colon (ulcerative colitis, Chron's disease, carcinomas and infections) whereby high local concentration can be achieved while minimizing side effects that occur because it reduce the release of drug in the upper GIT or unnecessary systemic absorption.

Flavonoids, a group of approximately 4000 naturally occurring compounds with a wide range of biological effects, including anti-ulcer activity, have been proposed to increase the content of mucosal prostaglandin and decrease histamine secretion from mast cells through histidine decarboxylase inhibition (Borrelli and Izzo, 2000).

Luteolin is soluble in ethanol, methanol, DMSO, and dimethyl formamide approximately 5, 10, and 20 mg/ml, respectively. Luteolin is sparingly soluble in aqueous buffers, slightly soluble in water. Anti-inflammatory, antioxidant and free radical scavenger action has been defined. It inhibits LPS-induced TNF- α , IL-6 and inducible nitric oxide production and blocks NF- κ B and AP-1 activation (Aziz et al., 2018; Odontuya et al., 2005). Antiproliferative effect produced through inhibits proliferation of Lewis lung carcinoma cells *in vivo*.

Luteolin is one of the most important bioactive flavonoids, which possesses potent neuroprotective effects. Luteolin also suppresses inflammation in the brain tissues and regulates different cell signaling pathways. Moreover, since oxidative stress and neuro-inflammation have a crucial role in the initiation and progression of neurodegenerative diseases and neuronal cell death, antioxidants and anti-inflammatory agents, such as luteolin, with nanofibrous scaffolds can be used as novel therapeutic agents for the treatment of neurodegenerative diseases (Funakoshi-Tago et al., 2011). The primary aim of treatment of ulcerative colitis is the reduction of pain and inflammation. Oral treatment of such diseases is limited due to the side effects of nonsteroidal anti-inflammatory drugs (NSAIDS). Use of natural flavonoid can minimize the side effect with prominent therapeutic benefits. Aim of present study was to investigate protective role of luteolin in ulcerative colitis in rats.

Materials and methods

Chemicals and reagents

Myeloperoxidase (MPO) Kit was purchased from Krishgen BioSystems, Mumbai, India. Antioxidant Kit was purchased from BIO Innovations, Mumbai, India. Luteolin, PEG 400, dextrin, Dextran Sulphate Sodium, Sulfasalazine, were purchased Sigma Aldrich Chemicals Pvt Ltd, Mumbai, India.

All other common reagents and chemicals were used of the analytical grade and commercially available.

Preparation and optimization of luteolin microspheres

The microspheres were prepared by solvent evaporation method (Joseph, 2015). The Luteolin and dextrin (1%, 5%, 10% & 20% w/v) were dissolved in ethanol (99%). This solution was dispersed in a 250 ml beaker in 100 ml of liquid paraffin light that contained different Span 80 concentrations. Then prepared dispersion was stirred at a speed of 400 rpm for 30 minutes. Microspheres were centrifuged after the stirring time, washed multiple times with n - hexane, ether, and finally with acetone. Dried at 50°C, the microspheres were stored in a desiccator. For all batches rpm was maintained at 200-500. All the batches are prepared and optimized for various parameters.

The completely different variables of the formulation and process i.e. polymer concentration, drug concentration, surfactant concentration, stirring speed and effect of temperature which could affect the preparation and properties of microspheres were identified and studied. The optimization was done on the basis of particle size and drug loading efficiency. In order to study the influence of experimental parameters on the preparation of microspheres various parameters were optimized like luteolin and surfactant concentration, stirring speed, stirring time, temperature, and drug polymer ratio.

Characterization of luteolin microspheres

Particle size analysis

Particle size of prepared microspheres was determined by optical microscopy method. Particle size plays important role in release of drug from microsphere. If size of microspheres is less than 500 m release rate of drug will be high, the microspheres ranging between 200m - 500m, release rate will be in sustained manner (Srivastava et al., 2005).

Drug entrapment

The drug entrapment efficacies of different batches of luteolin microspheres formulations were observed and shown in Table 5.6. The entrapment efficiency of drug loaded microsphere were determined by using dialysis bag and 200 mL mixture of ethanol and PBS (pH 7.4) in 1:1 (with 20% PEG 400) ratio was taken as dissolution medium for 3 hr and untrapped drug was released in the medium. Further, percentage drug entrapment in microsphere was determined by UV method at 348 nm. One mL of sample was taken from dialysis bag and dissolved in methanol at 50°C by adding 9 mL of methanol into 10 mL of volumetric flask. The solution was filtered and cooled at room

temperature (Gawde and Agrawal, 2012).

From filtrate 1 mL was taken and diluted to 10 mL with PBS : ethanol mixture and measured at $\lambda_{\max}$ 348 nm against reagent blank. Similarly untrapped drug in dialysis medium was estimated by transferring 1 mL of dialysis medium into 10 mL volumetric flask and volume made up to the mark with PBS: ethanol mixture.

Percentage yield

Percent yield is an important parameter of the formulation characterization. Percentage yield of different formulation was determined by weighing the Microspheres after complete drying (Chandiran et al., 2010).

Scanning Electronic Microscopy

Shape and surface characteristic of optimized luteolin microspheres were observed by Scanning Electronic Microscopy analysis. Using scanning electron microscope (JEOL, JSM-670F Japan), the formulated optimized batches of Luteolin microspheres were examined for shape and surface morphology. The optimized formulation sample of luteolin was finished on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 3.0 KV during scanning. Microphotographs were taken on different magnification and higher magnification (500X) was used for surface morphology (Sandeep et al., 2012).

In-vitro cumulative drug release study

In-vitro drug release study is a prerequisite for evaluating the *in-vitro* performance of a drug delivery system because the *in-vitro* drug release profile provides the most sensitive and reliable information for *in-vivo* evaluation that helps in ascertaining the future behavior of the designed formulation with regard to its drug release pattern and the time duration of its action in a biological system. All prepared formulations F1 – F4 of luteolin were subjected to dissolution studies. Dissolution study is carried out in USP type II apparatus at 50 rpm in the volume of 900ml dissolution medium was simulated gastric fluid, simulated intestinal fluid and simulated colonic fluid for 24 hours. On the basis of formulations process variables and cumulative release study, the optimized microspheres formulation F1 was selected.

Stability studies

Stability can be defined as the ability of a specific container / closure system to remain unchanged within its physical, chemical, microbiological, therapeutic as well as toxicological specifications (Pai et al., 2015). Stability of Dextrin based microspheres formulations on storage is a great concern as it is the major resistance in the development of marketed preparations. The prepared formulations were tested for stability

on storage them at room temperature and accelerated temperature (Pai et al., 2015).

The effect of temperature and humidity on the Dextrin based luteolin microspheres formulation was evaluated for 3 months under different storage conditions. The prepared microspheres were filled into an amber glass bottle and flushed with nitrogen gas before being airtight with a rubber gasket.

Microspheres were then stored at freeze temperature $5\pm3^{\circ}\text{C}$, $25\pm2^{\circ}\text{C}/60\pm5\%$ RH and $40\pm2^{\circ}\text{C}/75\pm5\%$ RH (as per ICH guidelines). Analysis of sample was made for particle size and residual drug content after a period of 30, 60 and 90 days.

Effect of storage on particle size

Particle size of the formulations stored at $5\pm3^{\circ}\text{C}$, $25\pm2^{\circ}\text{C}/60\pm5\%$ RH and $40\pm2^{\circ}\text{C}/75\pm5\%$ RH was determined by using a Zetasizer (Malvern Instruments, England) after a definite period of time i.e. 30, 60 and 90 days.

Effect of storage on residual drug content

The stability of polymeric carrier during storage at different temperature was evaluated by determining residual drug content after storage for 30, 60 and 90 Days.

In vivo studies

Animal protocol

Wistar albino rats (150-200g) of either sex were selected for the experiment. They were housed individually in well-ventilated, temperature controlled ($26\pm2^{\circ}\text{C}$) animal room for seven days of period prior experiment. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and they were kept under standard environmental conditions of laboratory temperature and water *ad libitum*. The animals were maintain alternate cycle of darkness and light at 12 hours. The animals were fasted for at least 12 hours before the onset of experiment. The experimental protocols were approved by Institutional Animal Ethics Committee.

Dextran Sulphate Sodium (DSS) induced colitis

The administration of DSS contained in water causes haematochezia, body weight loss, shortening of the intestine, mucosal ulcers and neutrophil infiltration. Acute colitis is regarded to be induced but not obtained by innate immunity. On the other side, the chronic stage is reported to be caused by lymphocytes activated by the cytokines secreted from the activated macrophages (Jurjus et al., 2004).

In rats, ulcerative colitis was caused by adding DSS (Dextran Sulfate Sodium) to water bottles, resulting in a 3%

(w / v) solution (Hirata et al., 2001). Free access to water comprising 3 percent oral DSS for 7 days was provided to the cattle. For 7 consecutive days, all treatment regimens were continued. Drugs were administered once daily by oral gavage and suspended in Sodium CMC. Clinical activity results were evaluated on the 8th day and the animals were anesthetized with ether and blood was gathered for biochemical assessment through retro orbital puncture. Daily recorded body weight, consistency of stools and gross bleeding.

All animals were divided into 5 groups consisting 6 animals in each. Group I normal or untreated animals received saline solution. Group II is control received Dextran sodium sulfate (3%w/v in drinking water) + 0.9% saline at a dose of 50 ml/kg, group III received Dextran sodium sulfate (3%w/v in drinking water) + LUT suspension 25 mg/kg, group IV received Dextran sodium sulfate (3%w/v in drinking water) + Luteolin microspheres suspension. Group V received Dextran sodium sulfate (3%w/v in drinking water) + Sulfasalazine (500 mg/kg) suspension.

Assessment of colon damage by macroscopic scoring

A clinical score assessing weight loss, stool consistency, and colon bleeding as described by Cooper, divided by 3, quantified the score for disease activity (Niu et al., 2013).

Body weight loss was calculated as the percent difference between the original body weight (day 0) and the body weight on any particular day.

Estimation of biochemical parameters

Myeloperoxidase (MPO) assay

MPO activity was identified using an MPO detection kit using the O-dianisidine technique (Liu and Wang, 2011; Yang et al., 2012). Blood was gathered and centrifuged from the eyes. The MPO activity was evaluated at 460 nm by absorbance using a spectrophotometer (Shimadzu). MPO activity was described as the enzyme degrading 1 μ mol per minute at 37 μ C and expressed in units per liter of serum.

Determination of malondialdehyde (MDA) content

By Mihara and Uchiyama (1978), lipid peroxidation was evaluated as the colon's MDA content. In short, MDA's

colorimetric determination is based on the response of one reactive aldehyde molecule with two thiobarbituric acid molecules at low pH (2–3) and 45 min at a temperature of 95°C. By treatment with N-butanol obtained the resulting purple color and spectrophotometrically determined the absorbance at 532 and 520 nm. As a measure of colonic MDA content, the distinction in optical density between the two wavelengths was used. MDA's final value was depicted as protein nmol/mg.

Antioxidants assay

Catalase was estimated following the breakdown of hydrogen peroxide according to the method of Beers and Sizer (1952). Superoxide dismutase (SOD) was assayed according to Misra and Fridovich (1972) based on the inhibition of epinephrine autoxidation by the enzyme. Reduced glutathione (GSH) content was determined in the tissue by the method of Moron et al, (1979).

Statistical analysis

The data were expressed as mean standard deviation (SD). The statistical significance of the difference in each parameter among the groups was evaluated using one-way analysis of variance (ANOVA) followed by the multiple comparison test of Tukey–Kramer tests. Criterion for statistically significant difference was chosen to be at $P < 0.01$.

Results and discussion

Formulation preparation, optimization and characterization

Luteolin microsphere was prepared by solvent evaporation method through various different optimized parameters. The effect of different variables on characterization of microspheres was studied in prepared different batches F1 to F4. The optimization of the formulation was done on the basis of drug concentration, stirring speed, effect of temperature, surfactant concentration (Span 80) and polymer (dextrin) ratio.

For optimization of luteolin concentration in the microspheres were prepared with varying percentage of drug concentration from 25, 50, 75 and 100 mg, while

Table 1. Mean particle size, percentage drug entrapment and yield for Luteolin microsphere batches (F1 - F4)

Formulation code	Mean particle size (μ m)	Drug entrapment (% w/w)	Percent Yield (%)
F1	77.62 $\pm$ 2.60	75.62 $\pm$ 2.67	77.34 $\pm$ 2.35
F2	80.24 $\pm$ 2.07	79.28 $\pm$ 2.03	81.64 $\pm$ 2.61
F3	82.95 $\pm$ 2.61	89.05 $\pm$ 2.46	92.43 $\pm$ 2.67
F4	76.81 $\pm$ 2.08	81.24 $\pm$ 2.74	80.27 $\pm$ 2.40

Mean $\pm$ SD, (n=3)

keeping other variables constant. Optimization was done on the basis of drug loading and particle as dependent variables for luteolin microspheres given in table 1 and figure 1. It was found that particle size was increased in increasing drug concentration because more drug entrapped inside the microspheres and drug loading was decrease, because after limited concentration of drug interacted with polymer's free active moiety.

For optimization of surfactant concentrations Span 80, microspheres were prepared using 0.5%, 0.75%, 1.0% and 1.25%w/v different concentration of surfactant in liquid paraffin while keeping the other parameters constant.

It was observed that, increase in surfactant concentration resulted in decrease in mean particle size, because when we higher the emulsifier concentration results in decrease in interfacial tension between inner aqueous and outer oily phase and it show smaller will be the mean particle size and size distribution. However this effect shows a plateau after which no significant effect of increase in surfactant concentration on particle size or size distribution is observed.

Figure 1. Particle size, percentage drug entrapment and yield for Luteolin microsphere batches (F1- F4)

An increase in concentration of surfactant has been shown to decrease drug entrapment. This is because of solubilizing effect of surfactant on drug during preparation of microspheres and subsequent loss of drug to external oil phase.

For optimization of Stirring speed, mechanical stirrer was varied from 200 to 500 rpm for both drugs microspheres preparation, while keeping the other variables constant. Stirring speed has been found to have significant effect on particle size. Stirring during addition of droplets and emulsification produces energy for dispersion of dextrin solution into droplets. With increasing the stirring speed particle size and drug loading found to be decreased. Which may be due to high stirring speed produced higher energy that provides higher surface area to loss of drug from outer surface of microsphere. This produce leaching effect of drug, so drug content in microsphere was decreased. Stirring speed of 200rpm has mean particle size 82.343.27 and drug loading was 90.284.65.

For optimization of temperature, the other variables kept constant. Microspheres formulations were prepared at different temperatures viz. 25°C, 37°C and 45°C. With increasing preparation temperature, the particle size of the microspheres increased. The main reason for increased microspheres size may be due to increased coalescence with higher preparation temperatures.

For optimization of polymer concentration is varied from 1- 20 % w/w by keeping all the variables constant. When the polymer concentration increased the particle size of the microspheres increased but after the certain concentration 10% w/w the particle size of microsphere was increased but the entrapment efficiency is decreased. So, on 10%, concentration

Table 2. Release study of Luteolin microspheres Batches F1- F4

Simulated Media (pH)		Cumulative % Drug release			
	Time in Hours	F1	F2	F3	F4
SIF 1.2	1	5.27	10.62	7.83	13.25
	2	8.06	21.27	12.81	23.51
SIF 6.8	4	45.26	41.28	24.08	36.75
SCF 7.4	8	58.40	56.84	48.93	66.27
	12	65.72	68.26	60.44	76.32
	16	78.06	72.34	74.53	85.04
	20	89.12	78.65	80.35	92.63
	24	99.47	89.47	87.33	98.61

Figure 2. SEM Image of Optimized Luteolin microsphere Formulation F1

of dextran, particle size was 89.05 ± 2.08 with good drug entrapment also.

For characterization of prepared microspheres various parameters like particle size, drug entrapment, percentage yield, shape and surface properties were analysed. Scanning electron microscopy of optimized formulation F1 was confirmed the spherical shape of microspheres and result shown in table 1, figure 1 and 2.

The *in-vitro* release study of Luteolin microsphere formulation and dissolution studies were carried out. Dissolution study was carried out using different dissolution medium like simulated

Table 3. Optimized parameters for Luteolin microspheres formulation

S. No.	Optimized parameters	Values
1	Drug concentration	25 mg
2	Surfactant concentration	0.5 (%w/v)
3	Stirring speed	200 rpm
4	Temperature	37 °C
5	Polymer concentration	10 (%w/w)

gastric fluid (SGF), simulated intestinal fluid (SIF) and simulated colonic fluid (SCF) for 24 hours.

Drug release study of F1 formulations was showed good release behavior in colon in comparison to stomach and intestine fluids. Because polymer dextrin is a polysaccharide which is only degraded by the enzymes presented in the colonic region (Table 2 and Figure 3).

The amount of drug release for the optimized formulation in first 5h studies showed that the polymer matrix remained intact in stomach and small intestine environment and retards the drug release from the matrix. There was an initial release of drug in the first 2-4 h of the studies indicating the un-entrapped drug on the surface of the matrix of the microsphere. To retard the drug release the optimized formulation was formulated with four different concentrations of dextrin (1% w/w, 5%w/w, 10%w/w, 20%w/w) and the drug release studies were performed for the optimized formulation (Table 3). Out of the four concentrations of dextrin solutions, formulation contain with 10%w/w showed good retardation and optimized release of drug for 4 h, while the 1%w/w, 5%w/w and 20%w/w formulations had low retardation and quick release. Once the formulation reaches the large intestine the dextrin coating gets easily degraded by the colonic enzymes and the drugs is directly exposed in colon.

The *in vitro* stability of luteolin microspheres were stored at

Figure 3. Release study profile of Luteolin microspheres formulation (F1 – F4)

Table 4. Effect of storage on particles size of Microspheres at different conditions

Storage Conditions	Particle size (µm) after at different duration			
	Initial	30 Days	60 Days	90 Days
5±3°C	83.61±1.52	84.82±1.07	86.10±1.31	87.34±1.41
25±2°C/60±5% RH	83.55±1.3	84.75±1.76	85.31±1.95	86.54±1.08
40±2°C /75±5% RH	84.05±1.54	85.31±1.27	86.65±1.67	87.25±1.34

Mean. ± SD, n=3

Table 5. Effect of storage on percent residual drug content of microspheres at different conditions

Storage Conditions	% Residual drug content at different duration		
	30 Days	60 Days	90 Days
5±3°C	99.82±2.04	98.64±1.30	97.45±1.55
25±2°C/60±5% RH	99.01±1.82	98.46±1.05	97.28±1.37
40±2°C /75±5% RH	99.74±1.62	97.52±1.88	96.43±1.33

Figure 4. Effect of storage on: (a) particles size of Microspheres at different conditions, (b) Percent residual drug content of microspheres at different conditions**Table 6.** Effect of luteolin and Luteolin microspheres on rat's colon

Groups	Disease activity Index (% protection)	Weight of colon (mg/cm)
Normal control (0.9% saline)	0	132.54±2.61
Control (0.9% saline+ DSS)	4.12±0.82	201.72±4.37
LUT + DSS	2.63±0.27 (55.23)*	138.2±3.4.06*
LUT Microsphere + DSS	1.53±0.62 (64.42)*	133.96±3.21*
Sulfasalazine (500mg/kg) + DSS	1.93±0.46(67.12)*	135.67±2.86*

n = 6 albino rats per group, value represents Mean S.D. *P<0.01, when compared each treated group with control group.

5±3°C, 25±2°C/60±5% RH and 40±2°C /75±5% RH. Changes in particle size after time interval of 30, 60 and 90 days were determined and results are shown in table 4.

No significant change in particle size was observed for drug containing microspheres after 90 days of storage at 5±3°C whereas at 40±2°C /75±5% RH a marginal increase in particle size was observed. It is evident that increase in average particle size was more pronounced when stored at accelerated temperature in comparison to refrigerated conditions (Table 5 and Figure 4). This may be attributed to the temperature induced fusion and aggregation of Microspheres at higher temperature.

The therapeutic effect of the formulation depends on the amount of drug that has been incorporated in the formulation; hence the residual drug content was monitored and compared with that obtained before stability testing period. The percent residual drug content of the microspheres was determined periodically after storing the formulations at refrigerated (5±3°C) and room temperature (25±2°C).

The data clearly suggested that refrigerated conditions are more suitable for the storage of microspheres formulations as after three months the residual drug content estimated. Conclusively, the formulations should be stored at low temperature preferably in the refrigerator for better stability.

In vivo studies

DSS produces severe macroscopic edematous inflammation in the colon. The disease activity index and wet colon weight for different groups were observed. The disease activity index and weight of colon for colitis control group were found as 4.12±0.82, 201.72±4.37, respectively. The disease activity index and colon weight for LUT and LUT microsphere treated groups of animals were observed significantly decreased as 2.63±0.27 and 1.53±0.62. LUT Microsphere decreases weight of colon significantly as 133.96±3.21 (Table 6, Figure 5). However, the LUT Microsphere showed better results for disease activity

Figure 5. Effect of Luteolin and Luteolin microspheres on: (a) Activity Index, (b) weight of rat's colon

Table 7. Effect of Luteolin and Luteolin microspheres on MPO and MDA level of colonic tissues of DSS induced colitis in rats

Treatment groups	MPO (OD/g tissue)	MDA (OD/g tissue)
Normal control (0.9% saline)	32.57±1.65	39.45±1.05
Control (0.9% saline+ DSS)	58.26±1.08	72.43±1.52
LUT + DSS	39.45±1.52*	47.28±1.62*
LUT Microsphere + DSS	33.75±0.89*	40.25±1.05*
Sulfasalazine (500mg/kg) + DSS	33.86±1.10*	39.52±1.41*

Values are presented as mean of optical density (OD) ± SD, *P<0.05, represent significant value compared with control group

Table 8. Effect of Luteolin and Luteolin microspheres on antioxidants level of colonic tissues in rats

Groups	Antioxidants level		
	SOD(μg/50 mg tissue)	CAT(μmol/50 mg tissue)	GSH(μmol/50 mg tissue)
Normal control (0.9% saline)	38.52±1.24	20.48±0.62	41.63±1.28
Control (0.9% saline+ DSS)	17.63±1.05	10.54±0.48	23.84±1.62
LUT + DSS	29.14±0.85	16.73±0.67	32.85±1.74
LUT Microsphere + DSS	36.51±1.02	18.62±0.95	38.16±1.05
Sulfasalazine (500mg/kg) + DSS	37.28±1.37	19.37±0.88	39.55±1.75

n = 6 albino rats per group, value represents Mean S.D. *P<0.01, when compared each treated group with control group

index and weight of colon, indicating its potent activity due to increased bioavailability of drug at the site. These data were also comparable to the standard drug treatment group.

The effect of Luteolin and their microspheres on different biochemical parameters were also observed. In the experiment, it was found that MPO activity was correlated with the development of colonic inflammation. DSS induced colitis significantly elevated MPO activity, whereas administration of LUT and their microsphere strongly inhibited MPO activity in rats that was comparable to the standard drug (Table 7 and Figure 6). The results of MDA level in tissue also indicated that colonic content of MDA decreased significantly and similar to the standard drug when compared

to the DSS control group. Treatment with Luteolin and luteolin microsphere exerted, to some extent, effects on reducing the colonic MDA level compared to animals that received DSS alone.

The effect of Luteolin and their microsphere on the various antioxidant activities (SOD, CAT and GSH) were observed in table 8. Both group of treatment i.e. Luteolin and Luteolin microsphere able to restored up to the normal level of antioxidant parameters, that was confirmed the potent antioxidant effect of Luteolin.

The level of antioxidants in colon tissues were observed significant decreased in colitis control group, may be due increasing free radicals generation. This decreasing level of

Figure 6. Effect of Luteolin and Luteolin microspheres on: (a) MPO and MDA level of colonic tissues in rats, (b) antioxidants level of colonic tissues in rats

SOD, CAT and GSH was significantly increased in treatment group with Luteolin as well as Luteolin microsphere. This improvement was comparable to the standard drug treated group (Table 8 and Figure 6).

The free radical scavenging property of Luteolin plays a significant role in ulcer healing. Significant increase in GSH level and reduction in MDA level has also been revealed in extracts treated groups while investigating *in vivo* antioxidant activity.

Conclusion

In conclusion, the free radical scavenging property of Luteolin plays a significant role in ulcer healing. Significant increase in GSH level and reduction in MDA level has also been revealed in extracts treated groups while investigating *in vivo* antioxidant activity. Hence, the probable mechanism of healing of ulcerative colitis by luteolin may be attributed to antioxidant and free radical scavenging property. Another luteolin also has been reported as a strong anti-inflammatory agent that could be beneficial for the treatment of ulcerative colitis.

Conflict of interest

None

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