**Supporting Information**

# Multifunctional Chrysin-loaded Gallic Acid-Glycerol Monostearate Conjugate injectable hydrogel for targeted inhibition of hypoxia-induced NLRP3 inflammasome in ulcerative colitis

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**Table of Content**

## 1. Percentage antioxidant assay (DPPH assay)

The percentage antioxidant activity was evaluated by DPPH assay. The result showed relatively low free radical scavenging activity as compared to GA, GA-GMS conjugate and CR@GA-GMS hydrogel due to the low number of hydroxyl groups present in the chrysin as compared to gallic acid. Similarly, no added increase in percentage antioxidant activity was reported in CR@GA-GMS Hydrogel as compared to GA-GMS Conjugate due to the encapsulation of CR into the GA-GMS Hydrogel network (**Figure S1**).

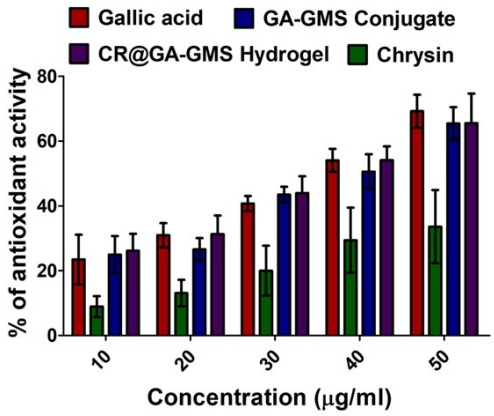


**Figure S1.** The percentage free radicle scavenging activity of GA, CR, GA-GMS hydrogel and CR@GA-GMS hydrogel.

## 2. Rheological study of the CR@GA-GMS hydrogel

The rheological property of CR@GA-GMS hydrogel was evaluated using a rheometer MCR 302 (Anton Paar, Austria) with the help of plate geometry PP-25. Amplitude sweep experiments were carried out with shear strain 0.1 – 500% and a constant angular frequency of 10 rad/s. Frequency sweep experiments were carried out in a frequency range of 0.1 – 100 rad $s^{-1}$ at 25 °C and 0.1% strain. Further, a thixotropy experiment was performed to evaluate the injectability of the CR@GA-GMS hydrogel at 25 °C in a frequency range of 0.1 – 100 rad $s^{-1}$.

## 3. Experimental Animal Ethical Statement

Female Swiss albino mice (22-25 g), 4-6 weeks old were purchased from the Central Animal Facility, National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab, India and kept according to the guidelines of Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA) in the animal house of National

Agri-food Biotechnology Institute (NABI), Mohali, Punjab, India under an ambient condition of 25 ± 1 °C with 12-hour light /dark cycles for acclimatization of about 1 week. They had free access to a standard rodent chew diet and water ad libitum. All animal experimentations were permitted by the "Institutional Animal Ethics Committee (IAEC)" of the Animal Experimentation Facility, National Agri-Food Biotechnology Institute (NABI), Mohali, Punjab, India which is fully accredited by the Committee for Purpose of Control and Supervision on Experimental Animals (CPCSEA) Chennai, India. The animal experimentation approval no. NABI/2039/CPCSEA/IAEC/2021/05.

**4. Fluorescent intensity of *in-vivo* and *ex-vivo* adhesion study**

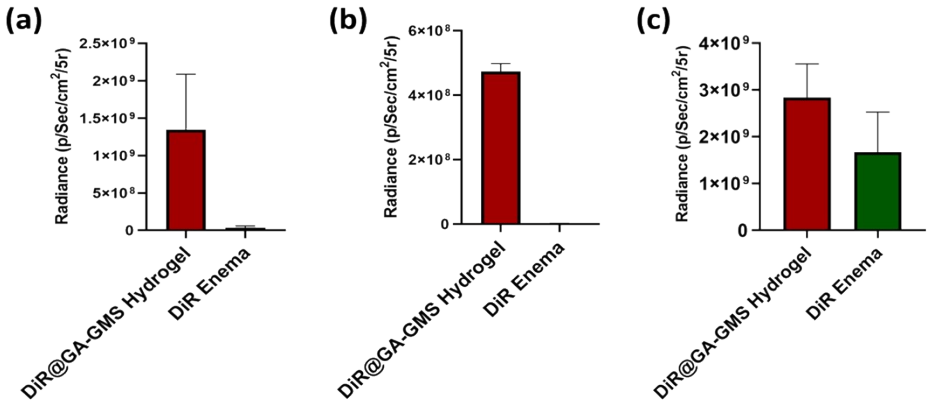


**Figure 2.** Fluorescence intensity graph of *ex-vivo* control (a), DSS-treated group (b) and *In-vivo* DSS-treated group (c)

**5. Study groups and treatment regimen**

Before the therapeutic study, the animals were given 4% DSS and after the development of UC, they were randomly divided into 6 groups of 6 mice in each group.

**Group I (Control)**: Mice received basal diet and purified water ad libitum.

**Group II (DSS)**: DSS induced colitis group, DSS (4%) in drinking water were given ad libitum for 5 days.

**Group III (GA-GMS Hydrogel)**: DSS was given as in group II, and 100 µl of GA-GMS Hydrogel was infused intrarectally (I.R.) on alternate days for up to 7 days with a total of 3 doses.

**Group IV (CR)**: DSS was given as in group II, and CR in 100 µl distilled water at the dose of 25 mg/kg body weight was infused I.R. on alternate days for up to 7 days with a total of 3 doses.

**Group V (CR@GA-GMS Hydrogel)**: DSS was given as in group II, and CR loaded GA-GMS Hydrogel (100 µl) equivalent to 25 mg/kg of CR, was infused I.R., on alternate days for up to 7 days with a total of 3 doses.

**Group VI (Safety)**: Treated as a control group with an infusion of 100 µl of GA-GMS Hydrogel I.R., on every alternate day for up to 7 days with a total of 3 doses.

The Animals were sacrificed (24 hr) after receiving the 3rd dose by following the guidelines of "Institutional Animal Ethics Committee (IAEC)" of the Animal Experimentation Facility, National Agri-Food Biotechnology Institute (NABI), Mohali, Punjab, India. The vital organs and colons were excised and stored at -80 degrees for further studies.

## 6. Disease Activity Index

The animals were visually inspected to show the symptoms to advertise the disease activity index (DAI).

| S. No | Group | Observation | | |
|---|---|---|---|---|
| | | Stool Consistency | Physical Activity | Rectal Bleeding |
| 1 | Control | 0 | 0 | 0 |
| 2 | DSS | 4 | 3 | 1 |
| 3 | GA-GMS Hydrogel | 0 | 0 | 0 |
| 4 | CR | 2 | 2 | 0 |
| 5 | CR@GA-GMS Hydrogel | 1 | 0 | 0 |
| 6 | Safety | 1 | 1 | 0 |

| Score | Stool consistency | Physical activity | Rectal bleeding |
|---|---|---|---|
| 0 | Normal Pellet (Hard) | Normal active (highly) | No bleeding |
| 1 | Soft Pellet | Mildly active | Bleeding |
| 2 | Loose watery stool | Low active | |
| 3 | Stickiness | Sedentary | |
| 4 | Diarrhea | | |

**Table 1.** Physical observations of various parameters on experimental animals over the study period. Values expressed in the table are average observational values.

## 7. Histological examinations

Various tissue staining techniques were used to evaluate the histoarchitecture of the colon tissue. In brief, after the completion of the study, colons were excised and washed with ice-cold normal saline and fixed in 10 % buffered formalin for a minimum of 24 hr. For making paraffin wax blocks, tissues were dehydrated in ascending grades of ethanol and finally in xylene for the total removal of water and embedded in paraffin wax. A tissue microtome was used to cut 5 µm thick sections from these paraffin blocks. These tissue sections were finally placed on slides and stained with different stains to evaluate the histoarchitecture of the colonic tissues.

## 8. Quantification of immune-positive cells in immunohistochemistry (IHC)

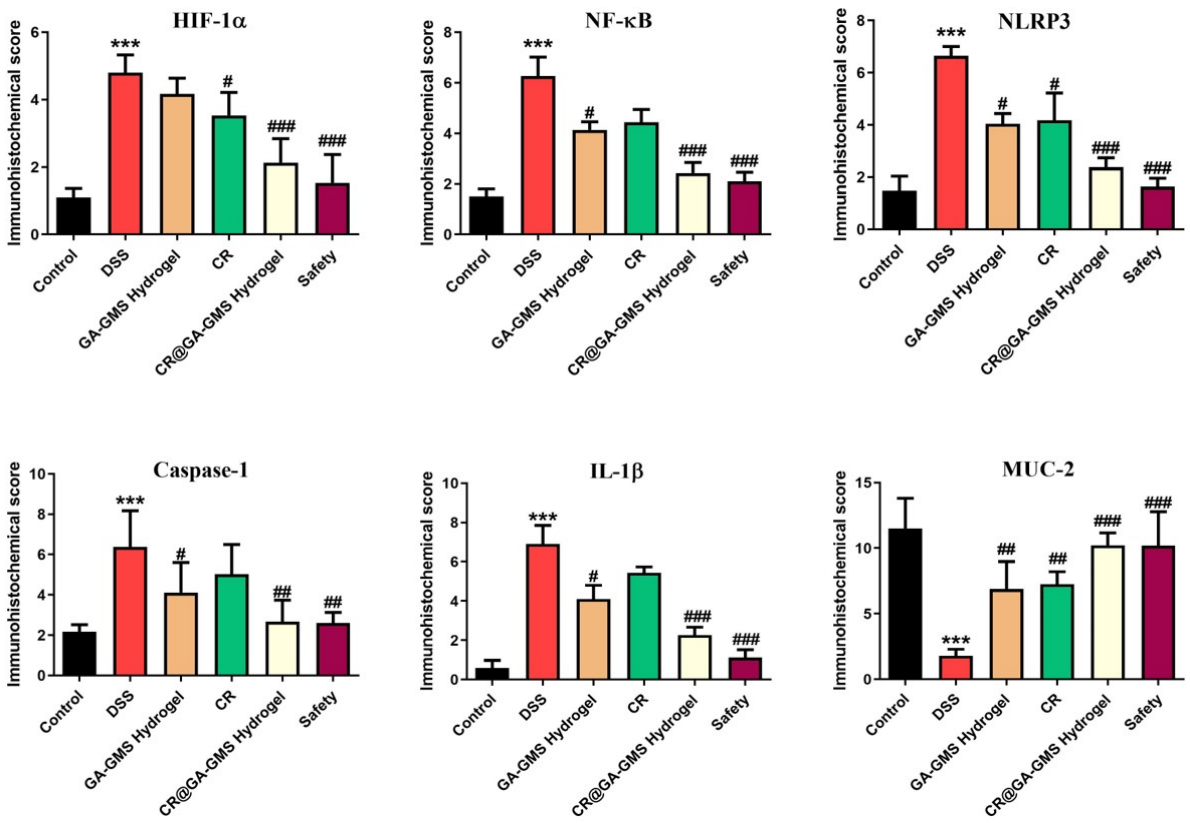


**Figure 3.** Semi-quantitative analysis of the expression of HIF-1α, Nf-kB, NLRP3, Caspase-1, IL-1β and MUC-2.

## 9. Serum toxicity markers

| Parameters | Groups | |
|---|---|---|
| | Control | Control+ GA-GMS hydrogel |
| **AST (IU/L)** | 34.12 ± 0.17 | 40.56 ± 1.22 |
| **ALT (IU/L)** | 51.78 ± 1.00 | 64.54 ± 2.01 |
| **Creatinine (mg/dl)** | 0.41 ± 0.05 | 0.57 ± 0.03 |
| **BUN (mg/dl)** | 11.65 ± 0.65 | 19.87± 0.71 |

## 10. Statistical analysis

In the present study, data was represented as mean ± SEM/SD and analysis was made by one-way and two-way ANOVA followed by Bonferroni multiple comparison test. The significant differences were represented as p-values *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ and #$p \leq 0.05$, ##$p \leq 0.01$, ###$p \leq 0.001$. Graph Pad Prism 5 and OriginPro 8.5 were used to perform statistical analysis.