Journal of Biomolecular Structure and Dynamics

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tbsd20

# Efficacy of green tea, its polyphenols and nanoformulation in experimental colitis and the role of non-canonical and canonical nuclear factor kappa beta (NF-kB) pathway: a preclinical in-vivo and in-silico exploratory study

Shoban Babu Varthya , Phulen Sarma , Alka Bhatia , Nishant Shekhar , Manisha Prajapat , Hardeep Kaur , Pugazhenthan Thangaraju , Subodh Kumar , Rahul Singh , Ashutosh Siingh , Ajay Prakash & Bikash Medhi

**To cite this article:** Shoban Babu Varthya , Phulen Sarma , Alka Bhatia , Nishant Shekhar , Manisha Prajapat , Hardeep Kaur , Pugazhenthan Thangaraju , Subodh Kumar , Rahul Singh , Ashutosh Siingh , Ajay Prakash & Bikash Medhi (2020): Efficacy of green tea, its polyphenols and nanoformulation in experimental colitis and the role of non-canonical and canonical nuclear factor kappa beta (NF-kB) pathway: a preclinical in-vivo and in-silico exploratory study, Journal of Biomolecular Structure and Dynamics, DOI: 10.1080/07391102.2020.1785946

**To link to this article:** https://doi.org/10.1080/07391102.2020.1785946

JOURNAL OF BIOMOLECULAR STRUCTURE AND DYNAMICS
https://doi.org/10.1080/07391102.2020.1785946



# Efficacy of green tea, its polyphenols and nanoformulation in experimental colitis and the role of non-canonical and canonical nuclear factor kappa beta (NF-kB) pathway: a preclinical in-vivo and in-silico exploratory study

Shoban Babu Varthya[a], Phulen Sarma[b], Alka Bhatia[c], Nishant Shekhar[b], Manisha Prajapat[b], Hardeep Kaur[b], Pugazhenthan Thangaraju[d], Subodh Kumar[b], Rahul Singh[b], Ashutosh Siingh[b], Ajay Prakash[a] and Bikash Medhi[b]

[a]Department of Pharmacology, All India Institute of Medical Sciences, Jodhpur, India; [b]Department of Pharmacology, Post Graduate Institute of Medical Education and Research, Chandigarh, India; [c]Department Experimental medicine and Biotechnology, Post Graduate Institute of Medical Education and Research, Chandigarh, India;[d]Department of Pharmacology, All India Institute of Medical Sciences, Raipur, India

Communicated by Ramaswamy H. Sarma

**ABSTRACT**

NF-kB plays a major role in the aetiopathogenesis of inflammatory-colitis. In this study, we evaluated the efficacy of green tea and its polyphenols and their nanoformulation in Tri-Nitro Benzene Sulfonic acid (TNBS) induced colitis in in-vivo system (Rat) and the involvement of non-canonical and canonical NF-kB pathway in green tea mediated protection (in-silico platform). We used the Wister rat model of TNBS-induced colitis. Rats were grouped into eleven groups (six animals each) and administered vehicle (ethanol), TNBS, Epicatechin (EC), Epigallocatechin (EGC), Epicatechin-gallate (ECG), Epigallocatechin-gallate (EGCG), sulfasalazine, green tea, EGCG + sulfasalazine, nano-EGCG and nano-EGCG + sulfasalazine for 14 days after induction of colitis. Colonic tissue was evaluated for the level of malondialdehyde, myeloperoxidase activity, catalase, reduced glutathione, glutathione peroxidase, IL-6, TNF-α, IL-1β, NF-κB and morphological and histopathological evidence of damage. In the in-silico part, molecular docking and dynamic simulation study of EGCG was done against different targets in NF-kB for detailed evaluation of the role of non-canonical and canonical NF-KB pathway. In our study, EGCG reduced colonic inflammation, markers of oxidative stress, TNF-α, NF-κB, IL-1β and IL-6. Nano-EGCG + sulfasalazine was more efficacious when compared to EGCG + sulfasalazine. In molecular docking and molecular dynamic simulation studies, EGCG showed a good binding profile to the inhibitor binding sites of IKK-beta, IKK-alpha and NIK. Thus, it can be concluded that EGCG showed protective action in experimental colitis acting through both non-canonical and canonical NF-kB pathway. Nano-EGCG + sulfasalazine combination showed better protection than nano-EGCG alone.

**Abbreviations:** IKK: Inhibitory Kappa Beta kinase; NF-kB: nuclear factor kappa beta; TNBS: Tri-Nitro Benzene Sulfonic acid; EGCG: Epigallocatechin-gallate; GT/GrT: green tea.

**ARTICLE HISTORY**
Received 13 May 2020
Accepted 17 June 2020

**KEYWORDS**
TNBS; colitis; green tea; Epigallocatechin-gallate; sulfasalazine; NF-κB

## Introduction

Inflammatory bowel disease (IBD) comprises of two relapsing and remitting intestinal inflammatory states namely Ulcerative Colitis (UC) and Crohn's disease (CD). NF-Kappa beta, a transcription factor, is a major regulatory component in intestinal mucosal immune-regulation and it plays a major role in the pathogenesis of IBD (Atreya et al., 2008; McDaniel et al., 2016; Rogler et al., 1998). NF-kB induces transcription and subsequent translation of many pro-inflammatory-cytokines and proteins that are crucial for colonic inflammation and tissue damage. The NF-kB pathway follows a vicious cycle. NF-κB itself gets activated by pro-inflammatory cytokines (Andoh et al., 1993; Li et al., 2013; Tak & Firestein, 2001; Verma et al., 1995). On the other hand, hyper-activation of the NF-κB axis results in over-expression of pro-inflammatory cytokines TNF-α, Interleukin- 1, 6, 12 and 23. These cytokines play the lead role in subsequent colonic inflammation and tissue damage (Atreya et al., 2008; Zaidi & Wine, 2018). In Crohn's disease, increased production of NF-kB correlates positively with histologic scores in biopsy (Han et al., 2017).

Intra-colonic administration of TNBS in Wister rat is a validated model of experimental colitis. TNBS activates T-helper cells and other inflammatory cells and thus leads to a colonic inflammatory state similar to IBD (Morris et al., 1989; Wirtz et al., 2007). The aetiopathogenesis of TNBS-induced experimental colitis is similar to IBD occurring in humans. Hence this model is considered as one of the best model of experimental colitis (Elson et al., 1995).

CONTACT Bikash Medhi drbikashus@yahoo.com Department of Pharmacology, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India

Supplemental data for this article can be accessed online at https://doi.org/10.1080/07391102.2020.1785946.

Polyphenols such as ECG (Epicatechin-gallate), EGC (Epigallocatechin), EC (Epicatechin) and EGCG (Epigallocatechin-gallate) (Andoh et al., 1993), present in Green tea are reported to exhibit antioxidant as well as anti-inflammatory activities (Verma et al., 1995). These polyphenols are reported to down-regulate NF-κB, TNF-alpha and TLR-4, which are implicated in the pathogenesis of IBD (Rahman et al., 2018). Green tea extracts primarily act by inhibiting TNF-α induction and thus inhibits the NF-κB pathway activation cascade (Wu et al., 1997). Green tea and its polyphenols exhibit anti-inflammatory properties and are potent inhibitors of leukocyte infiltration and pro-inflammatory cytokine surge (Baribault et al., 1994; Brown et al., 1995; Wu et al., 1997). One of the important green tea polyphenols is EGCG. EGCG has good oral bioavailability but it undergoes extensive glucuronidation (Lambert et al., 2004). However, PLA-PEG-encapsulated nanoformulation of EGCG increases bioavailability by 10-fold. PLA-PEG-encapsulated nanoparticles are biodegradable and environmentally friendly (Siddiqui & Mukhtar, 2010). In this context, we have evaluated the comparative efficacy of green tea and its different polyphenols (Epicatechin, Epigallocatechin and Epicatechin-gallate and Epigallocatechin-gallate) and nanoformulation of the most effective polyphenol (EGCG) as monotherapy and as combination therapy with sulfasalazine in TNBS-induced experimental colitis in Rats.

Many study have shown involvement of the NF-kB-pathway in EGCG mediated protection against experimental colitis (Abboud et al., 2008; Bing et al., 2017; Gerges Geagea et al., 2017; Ran et al., 2008), however, there is no data regarding whether canonical or non-canonical NF-kB-pathway is involved. In this context, we performed a detailed in-silico evaluation of the effective green tea polyphenols against different targets in the canonical and non-canonical NF-kB pathway. The first objective of our study was in-vivo evaluation of comparative efficacy of green tea, its polyphenols; nanoformulation of the most efficacious polyphenol alone and in combination with sulfasalazine in TNBS-induced Wister rat model of experimental colitis. The second objective of the study was in-silico mechanistic evaluation of the role of non-canonical and canonical NF-kB pathway.

## Materials and methods

### Experimental animals

Adult Wister rats were used for the study. Experimental animals of either sex, weighing 150–200 g were obtained from Advanced Small Animal Research Facility of the host institute. During the experiment, rats were kept in poly-acrylic cages (two animals in each cage). Before the experiment, rats were acclimatized to standard laboratory conditions (dark/light cycle of 12:12 h and temperature at 25 ± 2 °C) for 1 week. Animals were provided with clean drinking water and standard pellet diet ad-libitum. During the experiment the animals were maintained under same standard laboratory conditions. Approval from the Institutional Animal Ethics Committee (IAEC) was obtained (Reference No. 61/IAEC/354R).

### Design of experimental study

Animals were randomized into 11 groups (six animals in each group). The animals were grouped as control, disease control (TNBS group), Epicatechin (10 mg/kg, per os), Epigallocatechin (10 mg/kg, per os), Epicatechin-gallate (10 mg/kg, per os), Epigallocatechin-gallate (10 mg/kg, per os), sulfasalazine (360 mg/kg, per os), green tea (70 mg/kg, per os), Epigallocatechin-gallate (10 mg/kg, per os) in combination with sulfasalazine (360 mg/kg, per os), nano-EGCG and nano-EGCG + sulfasalazine for 14 days. All the groups received TNBS except group 1 (control) for model induction. In the Treatment started after 2 weeks of model induction. There was no restriction on feed and water for the animals. The doses of green tea polyphenols were selected from previous studies (Al-Malki & Moselhy, 2011; Byrav et al., 2011).

### Drugs

Epigallocatechin-gallate (EGCG), green tea, sulfasalazine, Epicatechin-gallate (EGC), Epicatechin (EC) and Epigallocatechin (EGC).

### ELISA kits

Kits for estimation of NF-κB, IL-6, IL-1 beta and TNF-alpha were procured from Ray Biotech.

### Induction of experimental colitis

Experimental colitis was induced following method by Morris et al. (1989). For preparing the TNBS solution, approximately 20 mg of TNBS was mixed with 0.25 mL of ethanol (35% V/V). Animals were anesthetized using ketamine 60 mg/kg. Following anesthesia, the TNBS solution was installed into the descending colon of rats using an infant feeding tube. In the case of control rats, only 35% ethanol (v/v) instilled.

### Preparation of nanoformulation of EGCG (PLA-Peg EGCG)

PLA-PEG-EGCC nanoparticles were prepared following previously described method (Siddiqui & Mukhtar, 2010). Briefly, 150 μL PLA-PEG (80 mg/mL in DMSO) was added to 100 μL EGCG (100 mg/mL DMSO). The product so formed was added to 20 mL of 2% (w/v) polyvinyl alcohol solution. A magnetic stirrer was used for stirring the solution for 3 h. The solution was dialyzed to remove the non-encapsulated EGCG.

### Evaluation of the severity of the disease

The extent of inflammation was measured using the disease activity index (DAI) (Cooper et al., 1993). To assess the DAI, the following parameters were evaluated: (a) body weight change (b) stool and (c) bleeding. A total score ranging from 0 to 12, 0 indicates no disease and 12 indicate very severe colitis.

### Estimation biochemical markers

Rat colon was homogenized using 1 M Tris-HCl buffer solution (5 mM ethylenediamine tetra-acetic acid; pH 7.4)

following homogenization at 12,000 × g for 45 min at 4 °C. Different biochemical parameters were evaluated using the supernatant.

### Lipid peroxidation (LPO)

LPO activity in the samples were evaluated using method described by Ohkawa et al. (1979). Lipid peroxidation generates Malondialdehyde (MDA) that reacts with thiobarbituric acid (TBA). The product generates red light absorbance at 532 nm. The aqueous TBA was mixed with acetate buffer (pH 3.5) and sodium-dodecyl-sulfate to form a solution. Rat tissue homogenate (10% w/v) was added to this solution. The solution was heated at 95 °C for 60 min. N-butanol-pyridine was used to extract the red pigment from the mixture. The absorbance was taken at 532 nm. External standard used was tetra-methoxy-propane. The final amount of MDA was indicated as nanomoles of MDA/mg protein (nmol/mg protein).

### Myeloperoxidase (MPO) activity

Neutrophils contains excess MPO. Increased MPO activity is associated with tissue infiltration by neutrophils. MPO activity was evaluated as previously described by Arab et al. (2014). Briefly, the colonic homogenate was centrifuged for 20 min at 20,000 × g (temperature 4 degrees). 0.167% of O-dianisidine hydrochloride and hydrogen peroxide was mixed with the supernatant. Finally, the absorbance was recorded at 460 nm for four minutes. The amount of MPO utilized in the conversion of 1 mM of $H_2O_2$ to water in 1 min is defined as one unit of myeloperoxidase activity.

### Glutathione peroxidase (GSH-Px)

We used method as described by Paglia and Valentine (1967). Hydrogen peroxide solution (0.1 mL; 2.2 mM) was added to the homogenate and finally absorbance was evaluated for 3 min at 340 nm. The concentration of the enzyme was measured as H2O2-consumed/min/mg homogenate.

### Assessment of reduced glutathione

The amount of reduced glutathione was estimated using the procedure described by Davies et al. (1984). After 2 min DTNB (0.01 M) was added and absorbance was evaluated at 412 nm. Reduced glutathione level was presented as μg GSH per-mg protein.

### Catalase (CAT)

Catalase is essential for the conversion of peroxide to water. We used the method by Lück (1965). After the addition of $H_2O_2$, the catalase activity was measured using the rate-of-change (delta) of absorbance/min at 240 nm and CAT-activity was indicated as μmol $H_2O_2$-consumed/min/mg protein.

### Pro-inflammatory cytokine levels estimation

In the present study, the serum levels of TNF-α, IL-1β and IL6 were analyzed using ELISA kits. The standard procedure was followed as per the instructions given in kits (Lehmann et al., 1999).

### Estimation of NF-κB

Estimation of NF-κB was based on the principle of solid-phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) as specified by Life science technologies (Sun et al., 2013).

### Gross morphology

Gross morphological damage was assessed as per criteria laid down by Morris et al. (1989), No damage was scored as 0, if there was localized inflammation without ulcer(s) then it was scored as 1, if there were ulcers along with normal adjacent tissue then it was scored 2, if there were ulcers with surrounding inflammation at single site then the score was 3, if 2 or >2 sites showed inflammation with or without ulceration then the score was 4 and if multiple lesions were present at different sites, or single lesion exceeding more than 1 cm then it was scored as 5.

### Histopathology

After excision of the colon, it was fixed in a 10% formalin solution for 24 h. The histopathologist examined the slides in a blinded fashion. Parameters evaluated under histopathology were colonic epithelium, dysplasia, edema, Crypt abscess, gland destruction, dilatation of glandular crypts, goblet cell-destruction and inflammatory-cell-infiltration. All the parameters were given a score between 0 and 3 and the range of sore being 0–24; higher the score than more severe is the disease state (Chen et al., 2007).

### In-silico docking and molecular dynamics study

Epigallocatechin-gallate was the most effective agent in decreasing colonic inflammation in TNBS-induced colitis in experimental animals. This specific phytochemical was further evaluated for possible binding to different targets in the NF-kB pathway [TNF-α (5MU8), IKK-Beta or IKK-2 (4KIK), IKK-Alpha or IKK-1 (5EBZ), NIK (4G3E) and NF-kB-P50-homodimer DNA-binding domain (1SVC) and NF-kB p52/RelB complex DNA-binding domain (3DO7)]. EGCG was further evaluated by molecular dynamics simulation studies for evaluation of the stability of the binding using Desmond (D E Shaw Research Group).

### Molecular docking studies: modeling platform

Molecular docking study of EGCG was conducted against selected targets [TNF-α, IKK-Beta or IKK-2, NF-KB inducing kinase (NIK), IKK-Alpha or IKK-1, NF-kB 50 homodimer DNA-binding domain and NF-kB p52/RelB complex DNA-binding domain]. For the molecular docking and MD simulations purpose, Schrodinger Maestro10.2 suit was used on an Acer

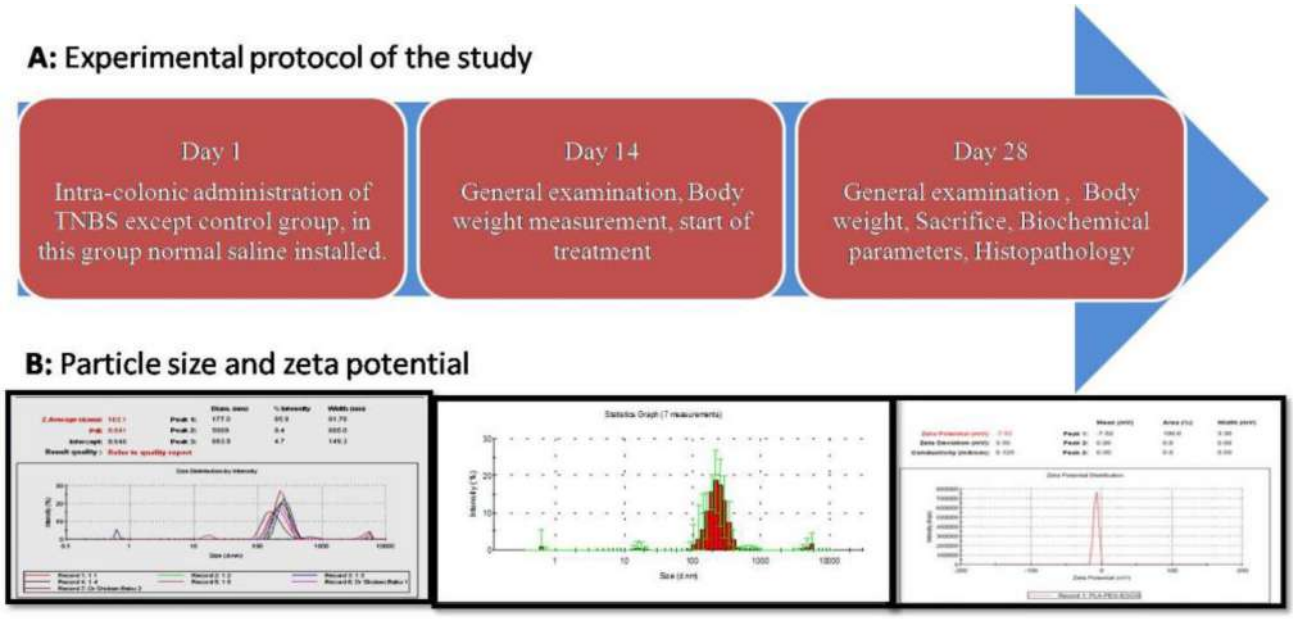


Figure 1. (A) Experimental protocol of the study. (B) Particle size and zeta potential.

predator laptop with an operating system consisting Linux Ubuntu OS 18.04.02 LTS platform.

### Retrieval of ligand and protein structure

In this study, Epigallocatechin-gallate (EGCG) molecules were retrieved from the PubChem (PubChem). 3D structure of target proteins [TNF-α (5MU8), IKK-Beta or IKK-2 (4KIK), NIK (4G3E), IKK-Alpha or IKK-1 (5EBZ), NF-KB-P50-homodimer bound to DNA (1SVC) and NF-kB p52/RelB complex DNA complex (3DO7)] were retrieved from PDB database (PDB Database).

### Preparation of the protein

While preparation of the target proteins, missing loops and side chains were added and water molecules were removed and the finally the structure was optimized and minimized using Protein preparation wizard tool (force field OPLS3) of Schrodinger Maestro (Desmond; Meng et al., 2011).

### Ligand preparation

LigPrep module (LigPrep) was employed to prepare the ligand structure of Epigallocatechin-gallate (EGCG). While prepared the ligands, we used OPLS3 forcefield and possible states were generated at target pH 7 ± 2. Desalting, tautomer generation and two stereoisomers generation was set per ligand (LigPrep).

### Molecular docking

To assess the binding profile of ligand Epigallocatechin-gallate with multiple targets, it was docked with the selected protein structures obtained from RCSB database [TNF-α (5MU8), IKK-Beta or IKK-2 (4KIK), NIK (4G3E), IKK-Alpha or IKK-1 (5EBZ), NF-kB-P50-homodimer DNA-binding domain (1SVC) and NF-kB p52/RelB complex DNA-binding domain (3DO7)], by using the glide receptor grid-based ligand docking program of Schrödinger Maestro suite. To estimate the binding affinity, the ligand was docked to the respective inhibitor binding sites on NFK-β, IkB kinase and TNF-α using Glide Extra precision (XP) function (Desmond; Meng et al., 2011).

### MD Simulation

Molecular dynamics study of EGCG was carried out against targets with favorable binding profile (TNF-α, NIK, IKK-α and IKK-β). MD simulations were carried out using Desmond module of Schrodinger (Desmond). Firstly, system was built using system builder with orthorhombic box type and box size calculation was done using buffer method and box volume was minimized. While building the system, SPC solvent model was used. First neutralization was done, which was followed by addition of salt. OPLS 2005 force field was used. This was followed by minimization with maximum iterations of 2000. The prepared model system is further evaluated in molecular dynamics studies using Desmond. A total simulation time of 25 ns was selected with recording interval trajectory of 25 ps with approximate 100 frames/ns. The model was relaxed before simulation. Stability was evaluated using RMSD for the Cα, RMSF and dynamic interaction profile between the ligand and target (Desmond).

### Experimental timelines

The detailed timeline of the experimental protocol is given in Figure 1(A).

### Statistical analysis

Normally distributed quantitative data were expressed as mean ± SEM and hypothesis testing was done using one way ANOVA and followed by post hoc Bonferroni test. Data

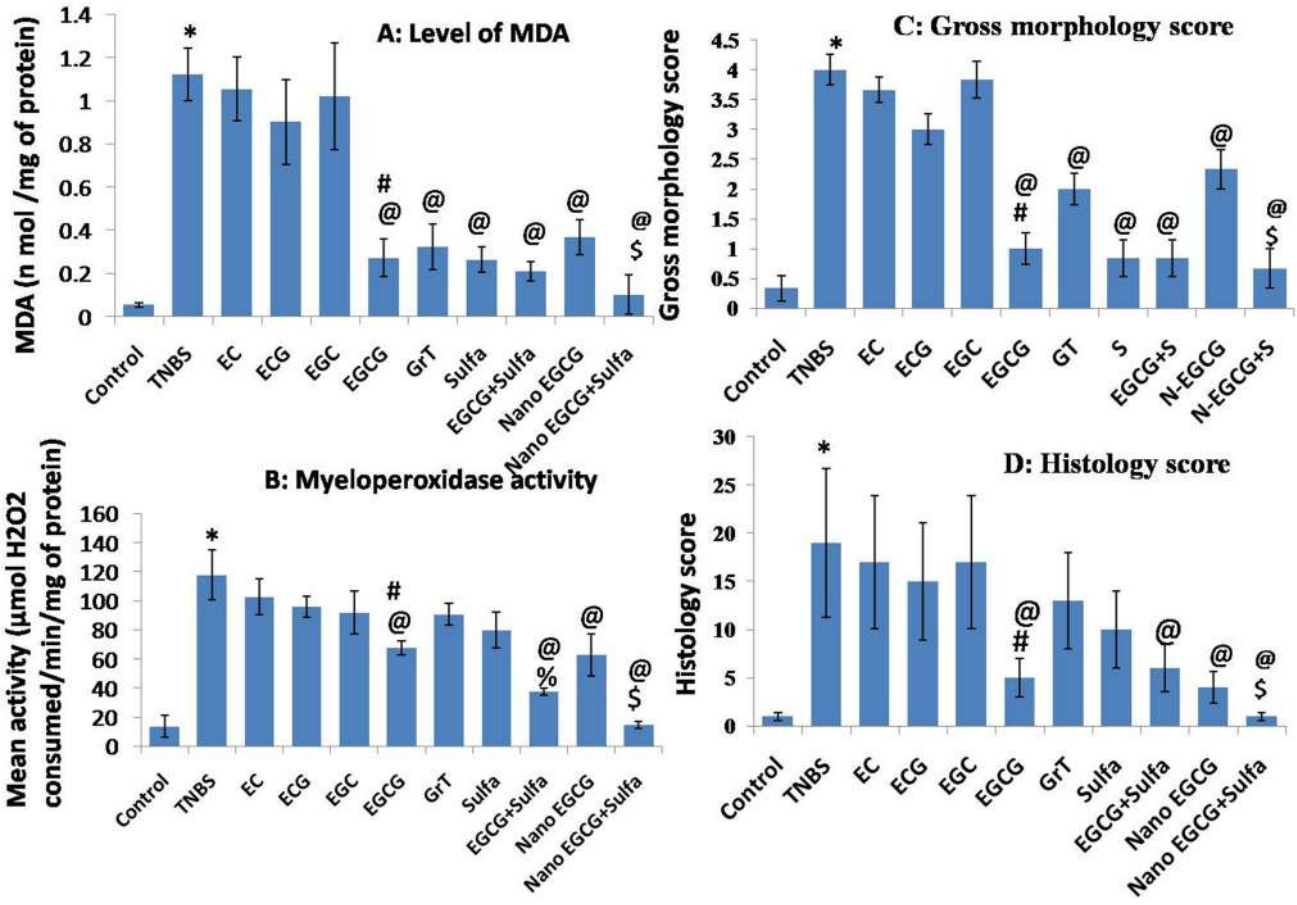


Figure 2. (A) Level of MDA, (B) myeloperoxidase activity, (C) gross morphology score, (D) histology score. *Statistically significant difference seen compared to control ($p < .05$), @Statistically significant when compared to TNBS (disease control) group ($p < .05$), #Statistically significant difference compared to other green tea polyphenols ($p < .05$), %Statistically significant difference between EGCG alone versus EGCG + S ($p < .05$). $Statistically significant difference between EGCG + S versus nano-EGCG + S ($p < .05$).

notfollowing normal distribution were represented as median (interquartile range) and statistical hypothesis testing was done by using one way ANOVA or Kruskal-Willis test as appropriate followed by post hoc intergroup comparison. SPSS version 20 software was used for the statistical part of the study. The value was considered as statistically significant when $p < .05$.

## Results

### Details of nanoformulation of EGCG

Particle size was measured using zeta sizer as shown in Figure 1(B). The average particle and molecular size was 163.1 nm, which was detected using dynamic light scattering, and zeta potential was −7.92 mV.

### Biochemical parameters

#### Lipid peroxidation

The disease control group (TNBS group) showed higher level of MDA than the control group. EGCG treatment led to a substantial decrease ($p < .05$) in the level of MDA than in the TNBS group. A significant-decrease in MDA level was seen in the nano-EGCG + sulfasalazine group when compared to EGCG + sulfasalazine group (Figure (2A)).

#### Myeloperoxidase activity (MPO)

In TBBS group, a considerable increase ($p < .05$) in MPO activity was observed than the control group. Compared to the TNBS group, EGCG treated animals showed considerably lower ($p < .05$) MPO activity. Nano-EGCG + sulfasalazine treated animals showed substantially lower ($p < .05$) MPO activity compared to the EGCG + sulfasalazine group (Figure 2).

#### Glutathione peroxidase

In the disease control group (TNBS group), the level of glutathione peroxidase was significantly lower when compared to the control group. When compared to other green tea polyphenols (EC, ECG and EGC) and the disease control (TNBS group), treatment with EGCG lead to significant improvement in glutathione peroxidase level. Compared to the EGCG + sulfasalazine group, treatment with the nano-EGCG + sulfasalazine was associated with higher tissue glutathione peroxidase level ($p < .05$) (Table 1).

Table 1. Effect of green tea on glutathione peroxidise (GPx), reduced glutathione and catalase activities.

| Groups | Content | | Activity |
|---|---|---|---|
| | GSH-Px (μmol of H2O2/min/mg of protein) | Reduced Glutathione (μg/mg protein) | Catalase (μmol of H2O2 consumed/ min/mg protein) |
| Control | 696 ± 24 | 29.4 ± 0.91 | 98 ± 2 |
| TNBS | 92 ± 6* | 4.44 ± 0.15* | 23 ± 3* |
| EC | 167 ± 23@ | 6.1 ± 1.17 | 28 ± 4 |
| ECG | 179 ± 28@ | 10.3 ± 0.8@ | 28 ± 6 |
| EGC | 236 ± 25@ | 7.7 ± 1.2@ | 33 ± 5 |
| EGCG | 348 ± 24@,# | 13.7 ± 0.4@,# | 68 ± 1@,# |
| Green Tea | 371 ± 38@ | 11.5 ± 0.44@ | 58 ± 2@ |
| Sulfasalazine | 404 ± 23@ | 12.4 ± 0.32@ | 61 ± 2@ |
| EGCG + Sulfasalazine | 493 ± 17@ | 18.55 ± 0.82@ | 84 ± 1@ |
| Nano-EGCG | 262 ± 11@ | 9.7 ± 0.4@ | 48 ± 1@ |
| Nano-EGCG + Sulfasalazine | 619 ± 16@,$ | 24.5 ± 3@,$ | 96 ± 1@,$ |

*Abbreviations:* TNBS, Tri-Nitro Benzene Sulfonic acid; EC, Epicatchin; ECG, Epicatchin gallate; EGC, Epigallocatchin; EGCG, Epigallocatchin-gallate.
Data expressed in mean ± SEM.
*Statistically significant difference seen compared to control ($p < .05$).
@Statistically significant when compared to TNBS (disease control) group ($p < .05$),
#Statistically significant difference between the polyphenols ($p < .05$).
%Statistically significant difference between EGCG alone versus EGCG + S ($p < .05$).
$Statistically significant difference between EGCG + S versus nano-EGCG + S ($p < .05$).

### Reduced glutathione

In TNBS group (disease control), a considerable decrease in the reduced glutathione level was seen ($p < .05$) than the control group. Compared to other green tea polyphenols and the disease control group, level of reduced glutathione was higher in the EGCG group ($p < .05$). Compared to the EGCG + sulfasalazine group, treatment with nano-EGCG + sulfasalazine was associated with significantly higher reduced glutathione level ($p < .05$) (Table 1).

### Catalase activity

Catalase activity was lower in the TNBS group ($p < .05$) than the control group. However, treatment with EGCG led to higher Catalase activity than the disease control (TNBS) group and other polyphenols ($p < .05$). Nano-EGCG + sulfasalazine treatment showed higher Catalase activity in comparison to the EGCG + sulfasalazine group (Table 1).

### TNF-α, IL-1β and IL-6

#### IL-1β

High IL-1β level was seen in the TNBS group ($p < .05$) in comparison to the control. Treatment with EGCG resulted in significantly decreased level of this pro-inflammatory cytokine as compared to the disease control (TNBS) group and animals treated with other green tea polyphenols ($p < .05$). Compared to the EGCG group, the EGCG + sulfasalazine group showed significantly lower level of IL-1β ($p < .05$). Levels of IL-1β was significantly lower ($p < .05$) in nano-EGCG + sulfasalazine group than the EGCG + sulfasalazine group (Figure 3).

#### IL-6

In TNBS group, IL-6 levels increased considerably ($p < .05$) than the control group. In comparison to the TNBS group, treatment with EGCG significantly lowered the level of IL-6 ($p < .05$). Compared to EGCG alone, treatment with the combination of EGCG + sulfasalazine resulted in significantly reduced level of IL-6 in the combination group ($p < .05$). In comparison to the EGCG + sulfasalazine combination, the nano-EGCG + sulfasalazine combination treated animals resulted in significantly lower level of IL-6 ($p < .05$) (Figure 3).

#### TNF-α (pg/mL)

In the disease control (TNBS) group, TNF-α levels considerably increased ($p < .05$) than the control group. TNF-α level significantly decreased in the in the EGCG group than the TNBS group and other polyphenols ($p < .05$). Comparison of EGCG alone and EGCG + sulfasalazine group, the combination group showed substantial decrease ($p < .05$) in TNF-α level. TNF-α level decreased considerably ($p < .05$) in nano-EGCG combined + sulfasalazine group compared to the EGCG + sulfasalazine group (Figure 3).

### Levels NF-κB (pg/mL) in colonic homogenate

Level of NF-κB was considerably higher in the TNBS group ($p < .05$) than the control group. Level off NF- κB decreased significantly ($p < .05$) upon treatment with EGCG when compared to the TNBS group. In comparison to the EGCG alone treated group, the level of NF-κB decreased substantially ($p < .05$) in the EGCG + sulfasalazine group. The level of NF-κB considerably decreased ($p < .05$) in nano-EGCG + sulfasalazine group than the EGCG + sulfasalazine group (Figure 3).

### Gross morphology score

Among different polyphenols, treatment with EGCG resulted in less gross morphologic damage as indicated by significantly lower ($p < .05$) gross morphological scores in comparison to the TNBS group (disease control). There was substantially a decrease in gross morphology score in the

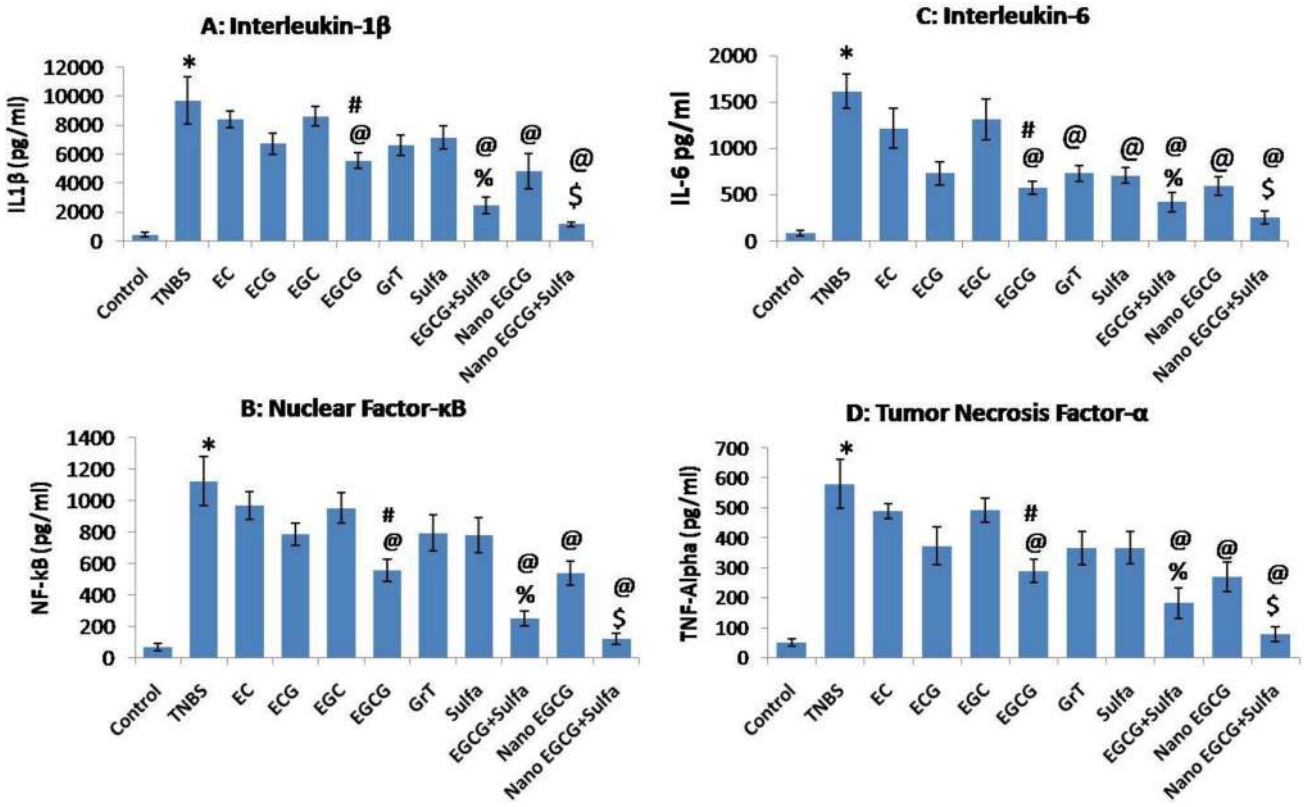


Figure 3. Cytokine profile (IL-1 beta, NF-KB, IL-6 and TNF-alpha) in different treatment arms. *Statistically significant difference seen compared to control ($p < .05$), [@]Statistically significant when compared to TNBS (disease control) group ($p < .05$), [#]Statistically significant difference compared to other green tea polyphenols ($p < .05$), [%]Statistically significant difference between EGCG alone versus EGCG + S ($p < .05$). [$]Statistically significant difference between EGCG + S versus nano-EGCG + S ($p < .05$).

nano-EGCG + sulfasalazine group than the EGCG + sulfasalazine group (Figures 2 and 4).

### Histopathology score

On day 28, average histological score of each group was shown in Figure 2. A higher histopathology score was seen in the TNBS group in comparison to the control ($p < .05$). EGCG group showed significantly lower ($p < .05$) histopathology scores than TNBS group. Nano-EGCG + sulfasalazine treatment resulted in significantly lower ($p < .05$) histopathology scores than the EGCG + sulfasalazine group (Figures 2 and 5).

### Molecular docking and MM-GBSA binding free energy

According to the Receptor-based docking, the ligand Epigallocatechin-gallate displayed varying binding profile with the target proteins [TNF-α (5MU8), IKK-Beta or IKK-2 (4KIK), NF-KB inducing kinase NIK (4G3E), IKK-Alpha or IKK-1 (5EBZ), NF-KB P50 homodimer DNA-binding domain (1SVC) and NF-kB p52/RelB complex DNA-binding domain] with GLIDE docking score of −7.92, −11.472, −9.045, −6.9, −4.125 and −5.030, respectively (data showed in Table 2). This implies that Epigallocatechin-gallate has a relatively efficient binding with the inhibitor binding site of IKK-Beta kinase (Figure 6). TNF-alpha, IKK-alpha and NIK (Supplementary Figures 1–5). Also, the post-docking MM-GBSA analysis revealed binding free energy of docked pose of the ligand, calculated −48.077 for TNF-alpha, −59.475 for IKK-beta, −45.206 for IKK-alpha, −49.843 for NIK and −38.217 for NF-KB P50 homodimer DNA-binding domain as shown in Table 2.

### MD (molecular dynamic) simulation studies

The root means square deviation (RMSD) of protein Cα backbone carbons along the trajectory provides an insight into the degree of dynamics the protein encounters in the presence of ligand, Epigallocatechin-gallate in this case. The average protein $C_\alpha$ RMSD for TNF-α (2.8 Å), IKK-Beta (3.0 Å), IKK-Alpha (3.0 Å) and NIK (2.5 Å) for 25 ns simulation directs the relative stability of the protein when subjected to the ligand EGCG. EGCG binding to TNF-α is primarily toward its solvent-soluble portion with very weak binding profile post-simulation. IKK-β is conjugated to EGCG via its N-terminal Kinase domain and reflects good interaction with the inhibitor. NIK protein has all its core kinase domain residues engaged with the inhibitor EGCG for most of the duration of the simulation. Protein IKK-α shows a similar nature as IKK-β and attracts the EGCG molecule at its kinase domain, with rich binding ratio. The above interpretation when aligned on to the plausible pathway, it suggests that inhibitory molecule EGCG interferes with NF-κB directed inflammatory response, by targeting and reducing the kinase activity of IKK-β in the canonical pathway. On the contrary, in the non-canonical

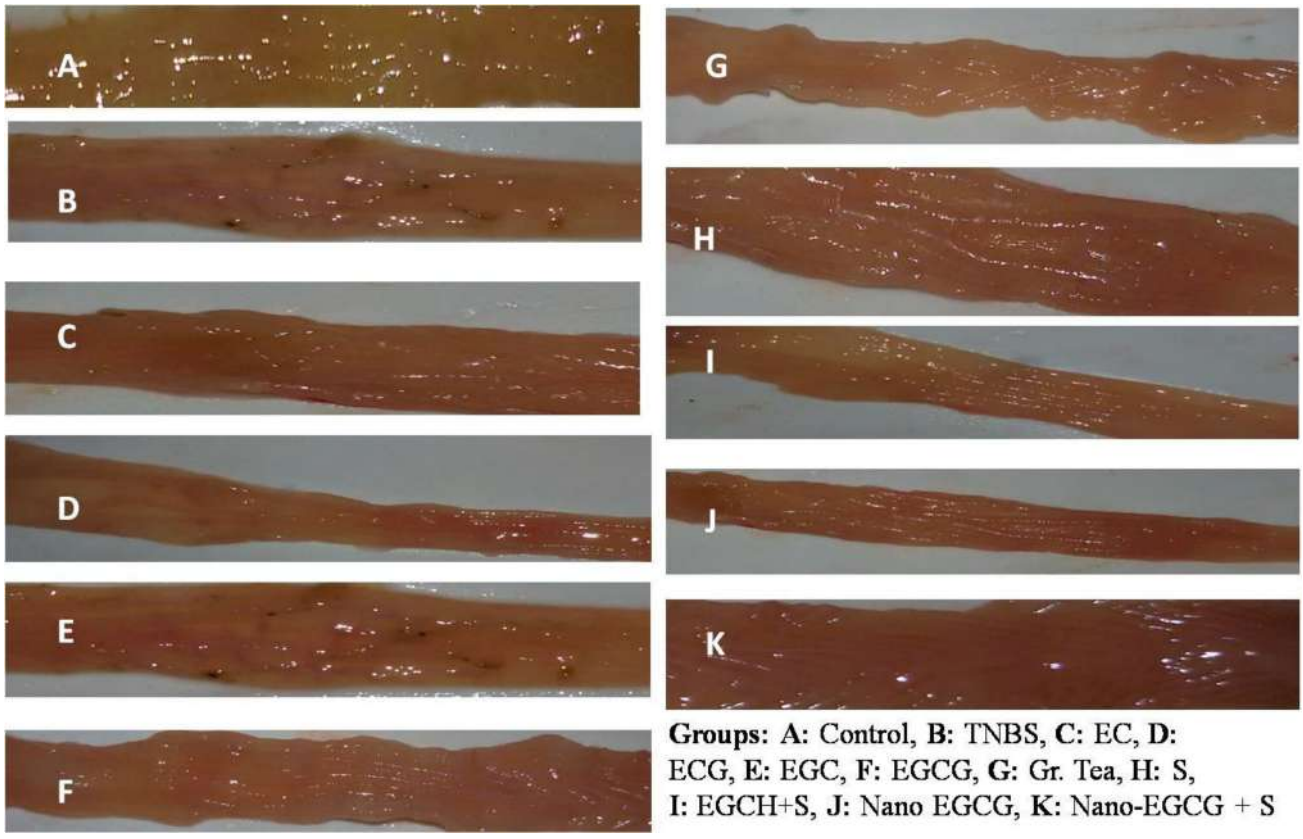


Figure 4. Gross morphology.

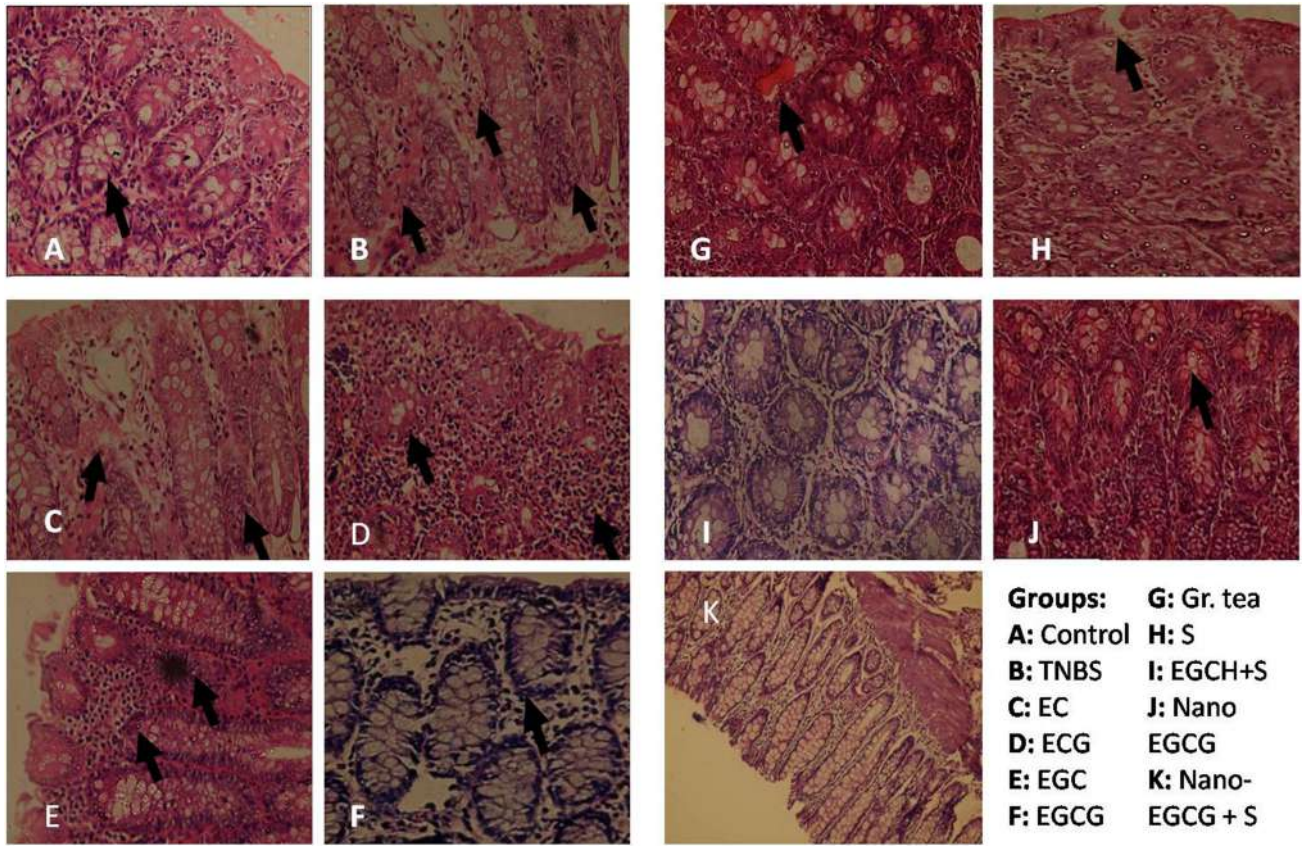


Figure 5. Histopathology.

Table 2. Details of target protein, ligands, docking score and MM-GBSA.

| Target protein (PDB id) | TNF-α (5MU8) | IKK-Beta or IKK-2 (4KIK) | IKK-Alpha or IKK-1 (5EBZ) | NF-KB inducing kinase NIK (4G3E) | NF-KB: DNA-binding domain, canonical pathway (1SVC) | NF-kB: DNA-binding domain, non-canonical pathway (3DO7) |
|---|---|---|---|---|---|---|
| Details of target protein structure | Human TNF-α in complex with JNJ525 | Human IkB kinase (IKK) beta with K252A | Crystal structure of human IKK1 | NF-kappa B inducing kinase (NIK) bound to cmp1. | NF-KB P50 homodimer bound to DNA | NF-kB p52/RelB/ DNA complex |
| Physiological role of the protein | Trigger in the canonical pathway, which subsequently activate IKK | In the canonical pathway, IKK-B is the main pathway responsible for IkB phosphorylation | In the non-canonical pathway, IKK-alpha plays a major role in the NF-KB pathway | Phosphorylate IKK-alpha and IKK-Beta | DNA binding of this domain lead to transcription of pro-inflammatory cytokines in canonical pathway | DNA binding of this domain lead to transcription of pro-inflammatory cytokines in non-canonical pathway |
| Bund inhibitor details | JNJ525 | K252a | 5TL $IC_{50}$: 1000 nM | Cmp1 $IC_{50}$: 4.2 nM | – | – |
| Grid generation site | JNJ525 binding site | K252a binding site | 5TL binding site | Cmp1 binding site | DNA-binding site | DNA-binding site |
| Ligand | Epigallocatechin-gallate (EGCG) | Epigallocatechin-gallate (EGCG) | Epigallocatechin-gallate (EGCG) | Epigallocatechin-gallate (EGCG) | Epigallocatechin-gallate (EGCG) | Epigallocatechin-gallate (EGCG) |
| Docking score (GLIDE) | −7.92 | −11.472 | −9.045 | −6.9 | −4.125 | −5.030 |
| MM-GBSA (kCal/Mol) | −48.077 | −59.475 | −45.206 | −49.843 | −38.217 | −59.147 |

pathway, both IKK-α and NIK show relevant selectivity for the EGCG molecule and are more likely to inhibit the NF-κB directed inflammatory response. Figures are produced in the supplementary material (Supplementary Figures 1–17).

## Discussion

Both UC and CD involves chronic inflammation of the digestive tract, but both these conditions are clinically and pathologically different.

### *Role of NF-KB*

NF-κB axis has a crucial role in the mechanism of IBD (McDaniel et al., 2016). NF-κB does so by causing transcription and subsequent translation of pro-inflammatory cytokines in colonic mucosa (Sun et al., 2013). The NF-kB family of transcription factors comprises of five inducible transcription factors [RelB, c-Rel, p50 (NF-kB1), p52 (NF-kB2) and p65 (RelA) (Liu et al., 2017)]. The interaction of NF-kB complex with specific DNA element kB enhancer results in transcription (Liu et al., 2017). Another important kinase complex involved in the NF-kB pathway is the IKK complex (Inhibitor of kB kinase) which comprises of one regulatory unit (IKK-gamma or NEMO) and two kinases (IKK-α and IKK-β) (Israël, 2010; Yang et al., 2001). The IKK-alpha and IKK-beta have catalytic properties (kinase), whereas the NEMO subunit is mainly regulatory in nature (Israël, 2010).

NF-kB activation has two distinct pathways (canonical and non-canonical pathway) (Israël, 2010). The IKK kinase complex stands at the center in both these pathways, making it the important target for therapies (Israël, 2010; Yang et al., 2001). Pro-inflammatory stimuli (e.g. TNF, IL-1, etc.) activates the canonical pathway and is a part of the innate response. The inhibitor of nuclear factor kappa beta (IkB) is subsequently phosphorylated by IKK-beta and the NEMO subunit (Israël, 2010). In the canonical pathway, NF-kB component heterodimers RelA/P50 is maintained in an inactive state in the cytosol, by IkB (endogenous inhibitor of NF-KB). Upon pathway activation by triggers (TNF-Alpha, IL-1, etc.), the IKK complex phosphorylates the IkB, which causes the release of RElA/P50 heterodimer (McDaniel et al., 2016) and subsequently, the cytoplasmic kB (RelA/P50 complex) migrates to the nucleus and promotes transcription. At the activation loop sites, phosphorylation of IKKβ activates IKK (Liu et al., 2013). In TNBS-induced experimental model of colitis, macrophages trigger inflammation by releasing TNF-α (Yang et al., 2001).

Another pathway of NF-kB activation is the non-canonical pathway (Israël, 2010; Polley et al., 2016). In this pathway, activation of NF-kB does not depend upon IkB degradation, rather it relies upon the processing of NF-kB2 precursor protein p100. NF-kB inducing kinase (NIK) is another central signal transduction protein. NIK triggers IKK-alpha which in turn phosphorylate p100. This results in p100 ubiquitination and causes degradation of the terminal IkB like structure in the C terminal end, which results in freeing and nuclear migration of the non-canonical NF-kB complex p52/RelB (Liu et al., 2017; Liu & Wang, 2011).

This heterodimer complex (RelA/P50 in case of canonical pathway and p52/RelB in case of the non-canonical pathway) of NF-KB enters into the nucleus to participate in the transcription and translation of diverse pro-inflammatory markers e.g. TNF-α, IL-1 and 6 (Atreya et al., 2008; Jobin & Sartor, 2000; McDaniel et al., 2016). These pro-inflammatory cytokines cause deleterious effects on the mucous membrane of the large bowel (Martín et al., 2005; Sklyarov et al., 2011). TNF-α has a direct influence on intestinal epithelial injury and IL-1B and IL-6 play synergistic activity with TNF-α in IBD progression (Cho et al., 2011). There are preclinical reports showing the effectiveness of inhibitor of NF-KB (i.e. DTCM-G and DHME-Q) in ameliorating TNBS associated colitis in rats highlighting the importance of this pathway in TNBS associated colitis rats (El-Salhy et al., 2014).

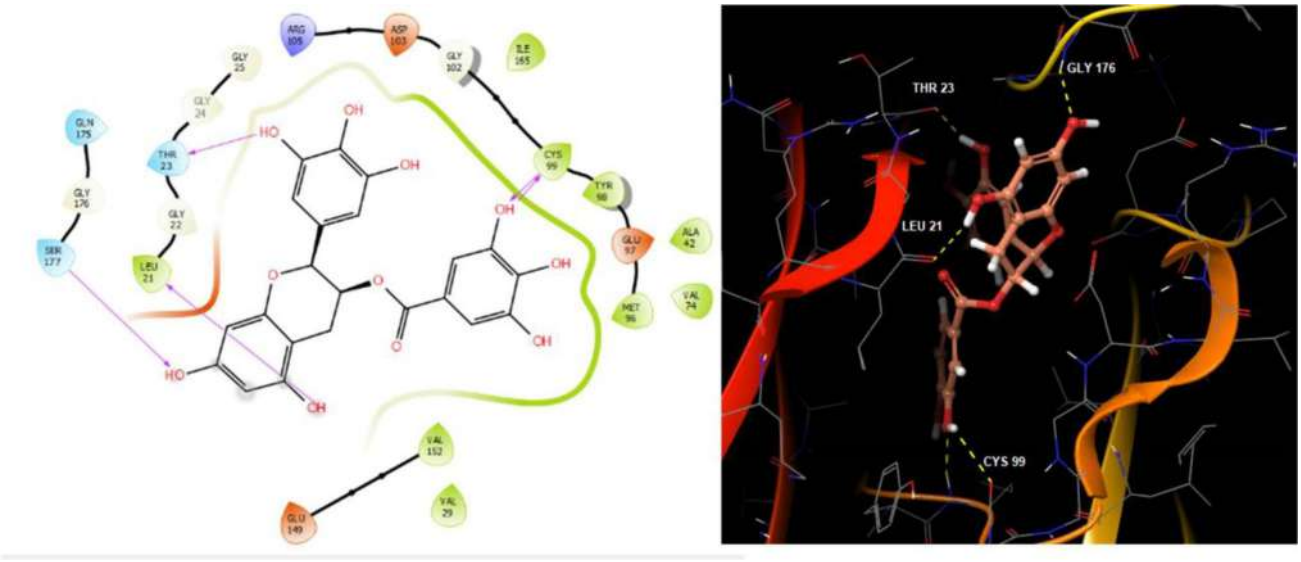


Figure 6. Molecular docking of EGCG with IKK-Beta (4KIK).

### TNBS-induced colitis

The study used the TNBS model of colitis. In the disease control group (TNBS group), the levels of catalase, GSH-Px and reduced glutathione were decreased statistically significantly than control. Lipid peroxidation markers (MDH, or LPO) were considerably increased in the TNBS group. Myeloperoxidase is an indicator of neutrophilic infiltration in the epithelium. In TNBS group the levels of myeloperoxidase substantially increased than the control group. Other parameters like pro-inflammatory cytokines and NF-κB levels were considerably higher in the disease control group (TNBS group). These results correlated well with histopathological findings. Gross morphological score and histopathological scores were higher in the disease control group (TNBS group). These findings highlight the validity of the model. These findings are in accordance with previous studies (Neurath et al., 2000; Rashidian et al., 2016).

### Efficacy of green tea polyphenols

Many natural products have shown efficacy in IBD (Bouzid et al., 2013; Moriasi et al., 2012; Tao et al., 2013). Green tea polyphenols are known to inhibit oxidized LDL-induced NF-KB activation (Wahyudi & Sargowo, 2007). Apart from inhibiting nuclear factor-κB, green tea and its polyphenols also show antioxidant properties. In the previous studies, they showed that green tea has the potential to control disease and improve the gross and histopathology of colon (Atreya et al., 2008; Han et al., 2017; Rahman et al., 2018; Rogler et al., 1998; Zaidi & Wine, 2018). In our study, among all the green tea polyphenol tested, only EGCG significantly decreased the inflammation as evidenced by low level of MDA, low MPO activity, low level of pro-inflammatory cytokines (IL-1 beta, IL-6, TNF-alpha), NF-kappa beta, higher level of glutathione peroxidase and reduced glutathione, low gross morphology, and histopathology score. This highlights the protective effect of EGCG in TNBS associated colitis.

### Nano-EGCG

Both EGCG and nano-EGCG were similar in terms of controlling disease activity and this was the first study to demonstrate this effect. However, compared to EGCG + sulfasalazine, the nano-EGCG + sulfasalazine was better in terms of controlling disease activity and resulted in near complete recovery by decreasing edema, inflammation, and bleeding. There were healed ulcers on colonic mucosa which explains that nano-EGCG controlled systemic inflammation and synergistic activity on local action. This synergistic effect of nano-EGCG can help in the management of moderate to severe IBD patients.

### Findings from in-silico simulation studies

As TNF-alpha (as a trigger for the NF-KB pathway and also as pro-inflammatory cytokine causing direct damage), IKK kinase complex (IKK-alpha, IKK-Beta), NIK and NF-KB crucial for inflammatory pathways in IBD, we screened the molecular docking properties of this EGCG against these targets. We selected PDB i.d. of these proteins bound to inhibitors in the nanomolar range. EGCG exhibited good binding profile to four proteins of the NF-KB pathway (TNF-alpha, IKK-Beta, IKK-alpha and NIK) in the inhibitor bound site with glide docking scores of −7.921, −11.422, −9.045 and −6.9, respectively. However, it's binding to NF-KB DNA-binding domain was not found satisfactory. These findings were further validated by molecular dynamics simulation studies. MD simulation assessment suggests that the molecule EGCG has the potency to inhibit the canonical and/or non-canonical pathway intermediates i.e. IKK-α, IKK-β and NIK which are essential modulators of NF-κB-mediated transcription. These findings highlight that EGCG act by inhibiting major pathway catalytic proteins in both the non-canonical and canonical NF-κB pathway. A graphical abstract highlighting the mechanism of protection EGCG against experimental colitis is showed in Figure 7. However, in-vitro and in-vivo validation of these findings are required.

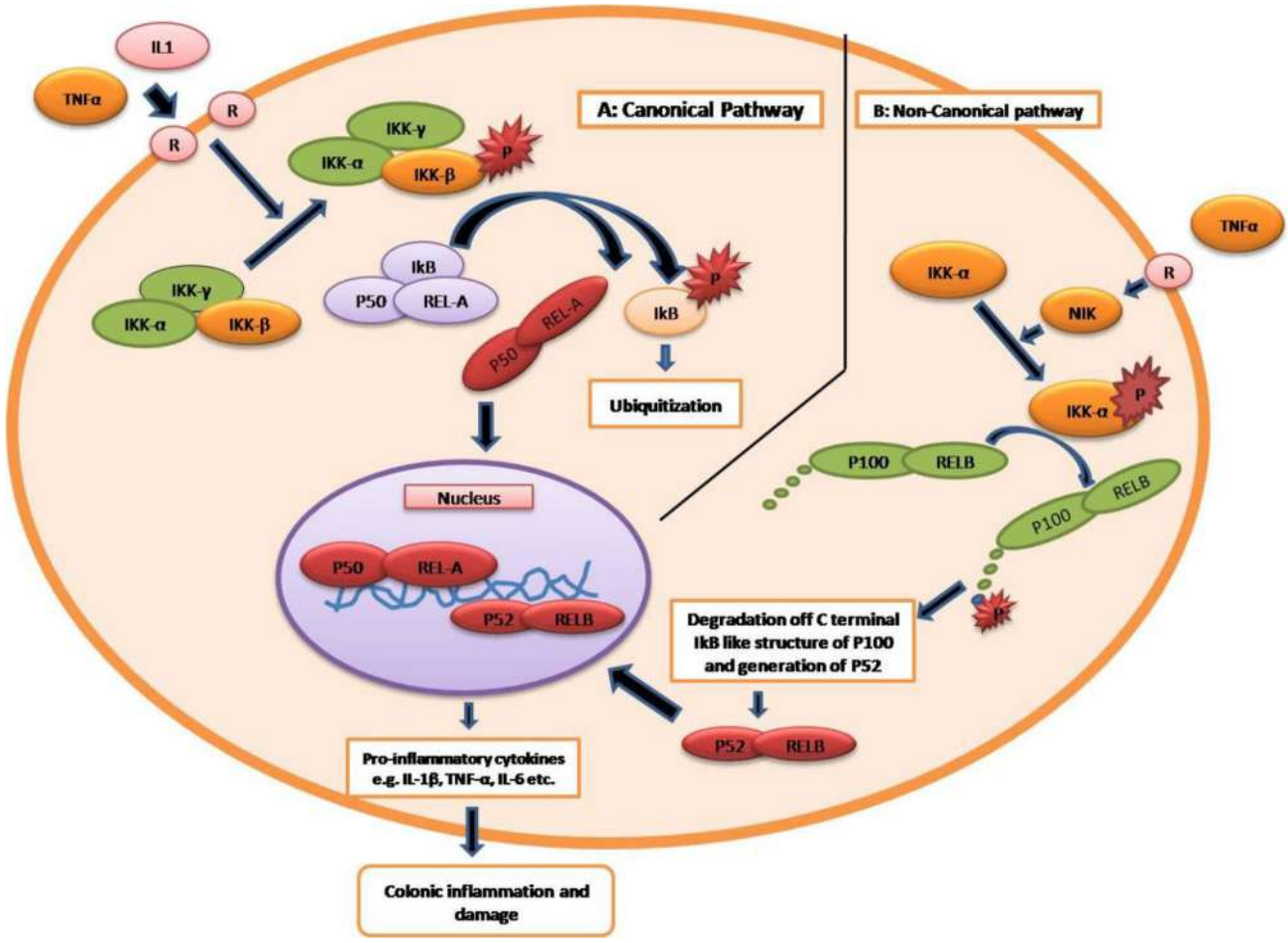


Figure 7. Graphical abstract: In the canonical pathway. (A) the trigger receptors for NF-kB pathway are activated by cytokines (e.g. TNF-α, IL-1, etc.). Upon activation, by these stimulus, the IKK complex gets activated by phosphorylation at the IKK-β site, which further phosphorylates IkB (inhibitor of kappa beta, IkB keeps P50 and RELA in cytosol by binding to it) and causes its degradation, releasing free P52-RELA dimer, which translocates into nucleus and causes transcription and translation of pro-inflammatory cytokines. In the non-canonical pathway (B), the pathway is activated by stimulation of TNF receptor, which stimulates NIK. NIK further phosphorylates IKK-α, which phosphorylates c terminal end of P100, causing its degradation and generation of P52 and resultant P52-RELB dimer translocates to nucleus. The targets of EGCG are showed in orange color (TNF-α, IKK-β, NIK, IKK-α). R: Receptor.

## *Major findings of this study*

1. Among all the green tea polyphenols tested, EGCG was most effective in reducing colonic inflammation.
2. Both EGCG and nano-EGCG have similar efficacy.
3. However, Combination of both formulation of EGCG with sulfasalazine showed that the nano-EGCG + sulfasalazine was better in controlling inflammation.
4. EGCG binds to inhibitor binding sites of TNF-alpha, IKK-α, IKK-β and NIK highlighting the possible mechanism of protection in TNBS associated colitis.

## Conclusion

Among all the green tea polyphenols, EGCG showed a protective effect against TNBS associated colitis. The efficacy of EGCG and nano-EGCG monotherapy was similar. However, the combination therapy of nano-EGCG + sulfasalazine was better than the EGCG + sulfasalazine combination. EGCG showed good binding affinity to the inhibitor binding site on TNF-alpha, IKK-alpha, IKK- beta and NIK, highlighting the role of non-canonical and canonical NF-Kb pathway in mediating the protective effect of EGCG against TNBS associated colitis.

## Acknowledgement

The authors acknowledge Mr. Nripendra Bhatta for his help.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Funding

Research Scheme, PGIMER, Chandigarh.

## ORCID

*Phulen Sarma* http://orcid.org/0000-0003-1926-7605
*Bikash Medhi* http://orcid.org/0000-0002-4017-641X

## References

Abboud, P. A., Hake, P. W., Burroughs, T. J., Odoms, K., O'Connor, M., Mangeshkar, P., Wong, H. R., & Zingarelli, B. (2008). Therapeutic effect of epigallocatechin-3-gallate in a mouse model of colitis. *European Journal of Pharmacology*, *579*(1–3), 411–417. https://doi.org/10.1016/j.ejphar.2007.10.053

Al-Malki, A. L., & Moselhy, S. S. (2011). The protective effect of epicatchin against oxidative stress and nephrotoxicity in rats induced by cyclosporine. *Human & Experimental Toxicology*, *30*(2), 145–151. https://doi.org/10.1177/0960327110369820

Andoh, A., Fujiyama, Y., Bamba, T., & Hosoda, S. (1993). Differential cytokine regulation of complement C3, C4, and factor B synthesis in human intestinal epithelial cell line, Caco-2. *Journal of Immunology (Baltimore, Md.: 1950)*, *151*(8), 4239–4247. https://doi.org/10.1016/0161-5890(93)90163-6

Arab, H. H., Al-Shorbagy, M. Y., Abdallah, D. M., & Nassar, N. N. (2014). Telmisartan attenuates colon inflammation, oxidative perturbations and apoptosis in a rat model of experimental inflammatory bowel disease. *PLoS One*, *9*(5), e97193. https://doi.org/10.1371/journal.pone.0097193

Atreya, I., Atreya, R., & Neurath, M. F. (2008). NF-kappaB in inflammatory bowel disease. *Journal of Internal Medicine*, *263*(6), 591–596. https://doi.org/10.1111/j.1365-2796.2008.01953.x

Baribault, H., Penner, J., Iozzo, R. V., & Wilson-Heiner, M. (1994). Colorectal hyperplasia and inflammation in keratin 8-deficient FVB/N mice. *Genes & Development*, *8*(24), 2964–2973. https://doi.org/10.1101/gad.8.24.2964

Bing, X., Xuelei, L., Wanwei, D., Linlang, L., & Keyan, C. (2017). EGCG maintains Th1/Th2 balance and mitigates ulcerative colitis induced by dextran sulfate sodium through TLR4/MyD88/NF-κB signaling pathway in rats. *Canadian Journal of Gastroenterology & Hepatology*, *2017*, 3057268. https://doi.org/10.1155/2017/3057268

Bouzid, D., Gargouri, B., Mansour, R., Amouri, A., Tahri, N., Lassoued, S., & Masmoudi, H. (2013). Oxidative stress markers in intestinal mucosa of Tunisian inflammatory bowel disease patients. *Saudi Journal of Gastroenterology*, *19*(3), 131–135. https://doi.org/10.4103/1319-3767.111956

Brown, K., Gerstberger, S., Carlson, L., Franzoso, G., & Siebenlist, U. (1995). Control of I kappa B-alpha proteolysis by site-specific, signal-induced phosphorylation. *Science*, *267*(5203), 1485–1488. https://doi.org/10.1126/science.7878466

Byrav, D. S. P., Medhi, B., Vaiphei, K., Chakrabarti, A., & Khanduja, K. L. (2011). Comparative evaluation of different doses of green tea extract alone and in combination with sulfasalazine in experimentally induced inflammatory bowel disease in rats. *Digestive Diseases and Sciences*, *56*(5), 1369–1378. https://doi.org/10.1007/s10620-010-1446-4

Chen, Y., Si, J-m., Liu, W-l., Cai, J-t., Du, Q., Wang, L-j., & Gao, M. (2007). Induction of experimental acute ulcerative colitis in rats by administration of dextran sulfate sodium at low concentration followed by intracolonic administration of 30% ethanol. *Journal of Zhejiang University Science B*, *8*(9), 632–637. https://doi.org/10.1631/jzus.2007.B0632

Cho, E-j., Shin, J.-S., Noh, Y.-S., Cho, Y.-W., Hong, S.-J., Park, J.-H., Lee, J. Y., Lee, J.-Y., & Lee, K.-T. (2011). Anti-inflammatory effects of methanol extract of Patrinia scabiosaefolia in mice with ulcerative colitis. *Journal of Ethnopharmacology*, *136*(3), 428–435. https://doi.org/10.1016/j.jep.2010.04.047

Cooper, H. S., Murthy, S. N., Shah, R. S., & Sedergran, D. J. (1993). Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Laboratory Investigation*, *69*(2), 238–249.

Davies, M. H., Birt, D. F., & Schnell, R. C. (1984). Direct enzymatic assay for reduced and oxidized glutathione. *Journal of Pharmacological Methods*, *12*(3), 191–194. https://doi.org/10.1016/0160-5402(84)90059-7

Desmond, S. Desmond | Schrödinger.

El-Salhy, M., Umezawa, K., Gilja, O. H., Hatlebakk, J. G., Gundersen, D., & Hausken, T. (2014). Amelioration of severe TNBS induced colitis by novel AP-1 and NF-κB inhibitors in rats. *The Scientific World Journal*, *2014*, 813804. https://doi.org/10.1155/2014/813804

Elson, C. O., Sartor, R. B., Tennyson, G. S., & Riddell, R. H. (1995). Experimental models of inflammatory bowel disease. *Gastroenterology*, *109*(4), 1344–1367. https://doi.org/10.1016/0016-5085(95)90599-5

Gerges Geagea, A., Rizzo, M., Eid, A., Hajj Hussein, I., Zgheib, Z., Zeenny, M. N., Jurjus, R., Uzzo, M. L., Spatola, G. F., Bonaventura, G., Leone, A., Massaad-Massade, L., & Jurjus, A. (2017). Tea catechins induce crosstalk between signaling pathways and stabilize mast cells in ulcerative colitis. *Journal of Biological Regulators and Homeostatic Agents*, *31*(4), 865–877.

Han, Y. M., Koh, J., Kim, J. W., Lee, C., Koh, S.-J., Kim, B., Lee, K. L., Im, J. P., & Kim, J. S. (2017). NF-kappa B activation correlates with disease phenotype in Crohn's disease. *PLoS One*, *12*(7), e0182071. https://doi.org/10.1371/journal.pone.0182071

Israël, A. (2010). The IKK complex, a central regulator of NF-kappaB activation. *Cold Spring Harbor Perspectives in Biology*, *2*(3), a000158. https://doi.org/10.1101/cshperspect.a000158

Jobin, C., & Sartor, B. R. (2000). NF-κB signaling proteins as therapeutic targets for inflammatory bowel diseases. *Inflammatory Bowel Diseases*, *6*(3), 206–213. https://doi.org/10.1002/ibd.3780060308

Lambert, J. D., Hong, J., Kim, D. H., Mishin, V. M., & Yang, C. S. (2004). Piperine enhances the bioavailability of the tea polyphenol (−)-epigallocatechin-3-gallate in mice. *The Journal of Nutrition*, *134*(8), 1948–1952. https://doi.org/10.1093/jn/134.8.1948

Lehmann, C., Egerer, K., Georgiew, A., Weber, M., Grune, T., & Kox, W. J. (1999). Inhibition of tumor necrosis factor-alpha release in rat experimental endotoxemia by treatment with the 21-aminosteroid U-74389G. *Critical Care Medicine*, *27*, 1164–1167.

Li, Y., Wang, S., Wang, Y., Zhou, C., Chen, G., Shen, W., Li, C., Lin, W., Lin, S., Huang, H., Liu, P., & Shen, X. (2013). Inhibitory effect of the antimalarial agent artesunate on collagen-induced arthritis in rats through nuclear factor kappa B and mitogen-activated protein kinase signaling pathway. *Translational Research*, *161*(2), 89–98. https://doi.org/10.1016/j.trsl.2012.06.001

LigPrep, S. LigPrep | Schrödinger.

Liu, S., Misquitta, Y. R., Olland, A., Johnson, M. A., Kelleher, K. S., Kriz, R., Lin, L. L., Stahl, M., & Mosyak, L. (2013). Crystal structure of a human IκB kinase β asymmetric dimer. *The Journal of Biological Chemistry*, *288*(31), 22758–22767. https://doi.org/10.1074/jbc.M113.482596

Liu, T., Zhang, L., Joo, D., & Sun, S.-C. (2017). NF-κB signaling in inflammation. *Signal Transduction and Targeted Therapy*, *2*, 17023 . https://doi.org/10.1038/sigtrans.2017.23.

Liu, X., & Wang, J. (2011). Anti-inflammatory effects of iridoid glycosides fraction of *Folium syringae* leaves on TNBS-induced colitis in rats. *Journal of Ethnopharmacology*, *133*(2), 780–787. https://doi.org/10.1016/j.jep.2010.11.010

Lück, H. (1965). Catalase. In H.-U. Bergmeyer (Ed.), *Methods of enzymatic analysis* (pp. 885–894). Academic Press.

Martín, A. R., Villegas, I., & Alarcón de la Lastra, C. (2005). The COX-2 inhibitor, rofecoxib, ameliorates dextran sulphate sodium induced colitis in mice. *Inflammation Research*, *54*(4), 145–151. https://doi.org/10.1007/s00011-004-1337-2

McDaniel, D. K., Eden, K., Ringel, V. M., & Allen, I. C. (2016). Emerging roles for noncanonical NF-κB signaling in the modulation of inflammatory bowel disease pathobiology. *Inflammatory Bowel Diseases*, *22*(9), 2265–2279. https://doi.org/10.1097/MIB.0000000000000858

Meng, X.-Y., Zhang, H.-X., Mezei, M., & Cui, M. (2011). Molecular docking: A powerful approach for structure-based drug discovery. *Current Computer-Aided Drug Design*, *7*(2), 146–157. https://doi.org/10.2174/157340911795677602

Moriasi, C., Subramaniam, D., Awasthi, S., Anant, S., & Ramalingam, S. (2012). Prevention of colitis-associated cancer: Natural compounds that target the IL-6 soluble receptor. *Anti-Cancer Agents in Medicinal Chemistry*, *12*(10), 1221–1238. https://doi.org/10.2174/187152012803833080

Morris, G. P., Beck, P. L., Herridge, M. S., Depew, W. T., Szewczuk, M. R., & Wallace, J. L. (1989). Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology*, *96*(3), 795–803. https://doi.org/10.1016/0016-5085(89)90904-9

Neurath, M., Fuss, I., & Strober, W. (2000). TNBS-Colitis. *International Reviews of Immunology*, *19*(1), 51–62. https://doi.org/10.3109/08830180009048389

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Paglia, D. E., & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal of Laboratory and Clinical Medicine*, *70*(1), 158–169.

PDB Database, R.P.D. RCSB PDB: Homepage.

Polley, S., Passos, D. O., Huang, D.-B., Mulero, M. C., Mazumder, A., Biswas, T., Verma, I. M., Lyumkis, D., & Ghosh, G. (2016). Structural basis for the activation of IKK1/α. *Cell Reports*, *17*(8), 1907–1914. https://doi.org/10.1016/j.celrep.2016.10.067

PubChem (−)-Epigallocatechin gallate. https://pubchem.ncbi.nlm.nih.gov/compound/65064

Rahman, S. U., Li, Y., Huang, Y., Zhu, L., Feng, S., Wu, J., & Wang, X. (2018). Treatment of inflammatory bowel disease via green tea polyphenols: Possible application and protective approaches. *Inflammopharmacology*, *26*(2), 319–330. https://doi.org/10.1007/s10787-018-0462-4

Ran, Z. H., Chen, C., & Xiao, S. D. (2008). Epigallocatechin-3-gallate ameliorates rats colitis induced by acetic acid. *Biomedicine & Pharmacotherapy*, *62*(3), 189–196. https://doi.org/10.1016/j.biopha.2008.02.002

Rashidian, A., Muhammadnejad, A., Dehpour, A.-R., Mehr, S. E., Akhavan, M. M., Shirkoohi, R., Chamanara, M., Mousavi, S.-E., & Rezayat, S.-M. (2016). Atorvastatin attenuates TNBS-induced rat colitis: The involvement of the TLR4/NF-kB signaling pathway. *Inflammopharmacology*, *24*(2–3), 109–118. https://doi.org/10.1007/s10787-016-0263-6

Rogler, G., Brand, K., Vogl, D., Page, S., Hofmeister, R., Andus, T., Knuechel, R., Baeuerle, P. A., Schölmerich, J., & Gross, V. (1998). Nuclear factor kappaB is activated in macrophages and epithelial cells of inflamed intestinal mucosa. *Gastroenterology*, *115*(2), 357–369. https://doi.org/10.1016/S0016-5085(98)70202-1

Siddiqui, I. A., & Mukhtar, H. (2010). Nanochemoprevention by bioactive food components: A perspective. *Pharmaceutical Research*, *27*(6), 1054–1060. https://doi.org/10.1007/s11095-010-0087-9

Sklyarov, A. Y., Panasyuk, N. B., & Fomenko, I. S. (2011). Role of nitric oxide-synthase and cyclooxygenase/lipooxygenase systems in development of experimental ulcerative colitis. *Journal of Physiology and Pharmacology: An Official Journal of the Polish Physiological Society* , *62*(1), 65–73.

Sun, P., Zhou, K., Wang, S., Li, P., Chen, S., Lin, G., Zhao, Y., & Wang, T. (2013). Involvement of MAPK/NF-κB signaling in the activation of the cholinergic anti-inflammatory pathway in experimental colitis by chronic vagus nerve stimulation. *Plos One*, *8*(8), e69424. https://doi.org/10.1371/journal.pone.0069424

Tak, P. P., & Firestein, G. S. (2001). NF-kappaB: A key role in inflammatory diseases. Journal of Clinical Investigation, *107*(1), 7–11. https://doi.org/10.1172/JCI11830

Tao, F., Qian, C., Guo, W., Luo, Q., Xu, Q., & Sun, Y. (2013). Inhibition of Th1/Th17 responses via suppression of STAT1 and STAT3 activation contributes to the amelioration of murine experimental colitis by a natural flavonoid glucoside icariin. *Biochemical Pharmacology*, *85*(6), 798–807. https://doi.org/10.1016/j.bcp.2012.12.002

Verma, I. M., Stevenson, J. K., Schwarz, E. M., Van Antwerp, D., & Miyamoto, S. (1995). Rel/NF-kappa B/I kappa B family: Intimate tales of association and dissociation. *Genes & Development*, *9*(22), 2723–2735. https://doi.org/10.1101/gad.9.22.2723

Wahyudi, S., & Sargowo, D. (2007). Green tea polyphenols inhibit oxidized LDL-induced NF-KB activation in human umbilical vein endothelial cells. *Acta Medica Indonesiana*, *39*, 66–70.

Wirtz, S., Neufert, C., Weigmann, B., & Neurath, M. F. (2007). Chemically induced mouse models of intestinal inflammation. *Nature Protocols*, *2*(3), 541–546. https://doi.org/10.1038/nprot.2007.41

Wu, G. D., Lai, E. J., Huang, N., & Wen, X. (1997). Oct-1 and CCAAT/Enhancer-binding Protein (C/EBP) bind to overlapping elements within the interleukin-8 promoter the role of Oct-1 as a transcriptional repressor. *The Journal of Biological Chemistry*, *272*(4), 2396–2403. https://doi.org/10.1074/jbc.272.4.2396

Yang, F., Oz, H. S., Barve, S., Villiers, W. J., de McClain, C. J., & Varilek, G. W. (2001). The green tea polyphenol (−)-epigallocatechin-3-gallate blocks nuclear factor-kappa B activation by inhibiting I kappa B kinase activity in the intestinal epithelial cell line IEC-6. *Molecular Pharmacology*, *60*(3), 528–533.

Zaidi, D., & Wine, E. (2018). Regulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ) in inflammatory bowel diseases. *Frontiers in Pediatrics*, *6*, https://doi.org/10.3389/fped.2018.00317.