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Effect of Aqueous Extract of *Trigonella Foenum-Graecum L.* Seeds On Acetic Acid- Induced Ulcerative Colitis In Rats

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Abstract

Ulcerative colitis is an inflammatory disorder affecting colonic mucosa, characterized by intense inflammation and mucosal damage. The currently available medical treatment is not completely safe and effective in alleviating the disease manifestations and its complications. The present study aimed to evaluate the protective effect of herbal product *Trigonella foenum-graecum* L. (TFG) seeds in acetic acid induced Ulcerative colitis (UC) in rats. The TFG extract was administered orally at two doses of 500 and 1000 mg/kg body weight. Compared to UC group, the TFG treated group showed significantly reduced severity of the disease as indicated by decreased Disease Activity Index (DAI) and decreased intensity of mucosal inflammation as indicated by macroscopic and histological scoring. Furthermore, biochemical assessment showed increased total protein, reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and decreased malonaldehyde (MDA) and pro-inflammatory cytokine TNF-α levels in TFG treated group compared to the UC group. The study demonstrates the beneficial effect of TFG in UC by its natural anti-inflammatory and antioxidant properties.

Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the gastrointestinal tract (S. V. Joshi et al., 2011). There are two main subtypes of IBD: Crohn's disease (CD) and Ulcerative colitis (UC) having a combined prevalence of 150–250/100000 population (Parkes & Jewell, 2001). The reported hospitalization was found to be 50.1 and 50.6 per 1 lakh population in CD and UC, respectively (Button et al., 2010).

Previous reports on the disease pathology of UC suggests that chronic inflammatory response in the colonic mucosa leads to tissue damage as a result of overproduction of reactive oxygen species (ROS) (Lih-Brody et al., 1996). The inflammatory response is amplified by the release of cytokines like TNF-α, IL-6 and IL-1β, which triggers the pathological response. The disease manifests as disabling intestinal and extra intestinal symptoms affecting the quality of life (Sartor, 1997).

Current medications for Ulcerative colitis are 5-aminosalicylic acid, corticosteroids, azathioprine, methotrexate, infliximab etc. These drugs are not specific and are associated with intolerable adverse effects as many of them are cytotoxic immunosuppressants and were given for prolonged periods owing to the chronic nature of the disease. Therefore, the development of newer drugs are essential to treat this disorder which has a long and complex pathogenetic history. Treatment with herbal products are believed to be safer and economical.

Trigonella foenum-graecum L. (TFG) is widely distributed throughout the world and belongs to the family Fabacecae. Traditionally in Ayurveda, this plant was found to be beneficial in many digestive conditions. Fenugreek seed is composed of biologically active compounds including flavanoids, amino acids, vitamins and alkaloids like, choline and trigonelline (Li et al., 2008). TFG is well known for its multiple pharmacological properties including antidiabetic, antioxidative, hypocholesterolemic, antineoplastic,

anti-inflammatory, antiulcerogenic, antipyretic, immunomodulatory and antitumor activity (Yadav & Baquer, 2014). Till date there are not enough studies to prove the beneficial effects of TFG in UC. The present study was designed to evaluate its potential benefits in UC model.

Materials And Methods

Drugs and chemicals

Sulfasalazine (SAZO, 500mg), was procured from Wallace Pharmaceuticals Private, Limited, India. *Trigonella foenum greacum* L. seeds was obtained from the local market in Udupi, Karnataka, India, which was authenticated by a botanist. All the Chemicals Sodium carbonate (Na2CO3), Sodium hydroxide (NaOH), Sodium-potassium alloy (NaK), Copper sulphate (CuSO4), Trichloro acetic acid (TCA), DTNB [5-5'-dithiobis (2-nitrobenzoic acid)], Potassium dihydrogen phosphate (KH2PO4), Disodium hydrogen phosphate (Na2HPO4), Sodium bicarbonate, Adrenalin bitartarate, Thiobarbituric acid (TBA), 2-propanol, chloroform, formalin, ethanol, paraffin wax, xylene, and DPX were purchased from Sigma Aldrich, St.Louis, MO, USA. Trizol Reagent, Primers- TNF α: Forward: 5' CACCATGAGCACGGAAAGCA 3', Reverse: 5' GCAATGACTCCAAAGTAGACC 3', GAPDH: Forward: 5' CAACTCCCTCAAGATTGTCAGCAA 3', Reverse: 5'GGCATGGACTGTGGTCATGA 3', cDNA synthesis kit and GoTaq Green mater mix were procured from Gennext Scientific and IT solutions, Mulky, India. All the solutions were freshly prepared and all the reagents used were of analytical grade.

Animals and ethics approval

The study was approved by institutional Animal Ethics Committee (**IAEC/KMC/93/2018).** Wistar rats were procured from the Central Animal Facility of the institute in accordance with the committee for the purpose of Control and supervision of experimentation on animal guidelines (CPCSEA). Thirty male albino wistar rats of weight ranging 150-250gm, aged around 8-10 weeks were used in the study. The animals were housed under standard condition, 12:12 light-dark cycle, 50% humidity and 28°C temperature and provided with standard food granules and water *ad libitum.*

Preparation of extract

Trigonella foenum-graecum L. aqueous extract was prepared as described by Noor (2007). Stock solution was prepared by boiling 10 gm of dried, grounded fenugreek seeds in 250 mL of double distilled water for 1 h. The extract was left all night and then filtered and made to 250 mL by double distilled water.

Experimental design

Thirty adult male rats were randomly allocated into five groups:

Group I- Normal control

Group II- UC: Acetic acid plus distilled water

Group III- Standard**:** Sulfasalazine 100 mg/kg plus acetic acid

Group IV- TFG- I (500mg/kg): *Trigonella foenum greacum* L. seed *extract* 500mg/kg plus acetic acid (D. V. Joshi et al., 2015).

Group V- TFG- II (1000mg/kg): *Trigonella foenum greacum* L. seed extract 1000mg/kg plus acetic acid (D. V. Joshi et al., 2015).

The dose of *Trigonella foenum greacum* L. seed extract was selected based on the anti- inflammatory property and toxicity study which demonstrated that there was no mortality rate till 5g/kg of *Trigonella foenum greacum* L. seeds (Muralidhara et al., 1999). Based on this we have selected two doses of *Trigonella foenum greacum* L. seed (500mg/kg and 1000mg/kg).

Induction of Ulcerative colitis

The drugs were administered per orally for 7 days, along with diet. The volume of drugs were kept constant at 5 ml/kg. Control group received distilled water. On day 8 following overnight fasting, UC was induced by administration of 1 ml of 4% acetic acid (AA) transrectally using an 8Fr (2.7 mm) soft paediatric catheter coated with lignocaine anaesthetic. The catheter was passed till 6-8 cm from the anus under low dose ether anaesthesia. The rats were maintained in head down position (trendelenburg position) after AA administration for 10 seconds to prevent any leakage. The acidic solution was aspirated out and it was followed by transrectal colon wash with 2 ml of phosphate buffer saline (PBS) at pH 7. Ulcerative colitis was induced in groups II to V. The normal control group received only normal saline transrectally. Twenty-four hours following induction of colitis, animals were sacrificed under anaesthesia.

Colon weight/ length ratio

Colon weight was measured in grams and length in centimetres. The ratio of weight in grams to length in cm was calculated by Aleisa et al. (2014).

Disease Activity Index (DAI)

DAI was assessed by Cooper et al. (1993) method. The changes in growth rate, stool consistency, and presence of gross bleeding or occult blood in feces was scored from 0~4 for each animal with a score 0 as normal stool consistency and no occult/gross rectal bleeding while the maximum score 4 with diarrhea, gross bleeding along significant decrease in growth.

Macroscopic Scoring

Colon from each animal was separated and dissected out for macroscopic scoring. Scoring was done according to Morris et al. (1989). A colon of length 4 cm extending proximally 2 cm above the rectum was dissected, split longitudinally on a piece of paper, and the colon was scored macroscopically with score 0 as normal gross morphology and 5 as intensive inflammation and ulceration[13].

Histopathology

The colonic tissues stored in 10% formalin, fixed in paraffin wax and washed in graded alcohol series. The tissue sections of 5µm thickness were taken and mounted on the slides. The staining was done by deparaffinization using xylene at different time intervals followed by hydration using a series of graded alcohol (100%, 90%, 80%, 50%). After hydration, the tissues were stained with hematoxylin, and the slides were subjected to washing by slow running water. Differentiation and blueing were done after the hematoxylin stain and the tissues were counterstained with 1% eosin. Later the slides were dehydrated with absolute alcohol and cleared with pure xylene. Following this, the tissue was mounted with Dibutylphthalate Polystyrene Xylene, and coverslip was placed. The slides were left for air drying at room temperature and examined under light microscope.

The microscopic colon damage was scored as described by Gálvez et al. (2001) with a score 0 being normal colon and a maximal score of 5 with transmural inflammation and intense ulceration.

Biochemical Assessment

Preparation of Homogenate

For the determination of tissue Total protein (TP), antioxidants and Lipid peroxidation (LP), 10% homogenate of colon was prepared with ice cold KCL (150mM) using homogenizer (Model:Yamato L.S.GL.H-21, Japan) and centrifuged at 3000 rpm for 10 min at 4°C.

Total protein

The total protein estimation is based on the reduction of Cu2+ to Cu+ by protein in an alkaline medium. Chelation of copper (cupric ion) with protein in alkaline environment form a light blue chelate complex between peptides of three or more amino acids with cupric ion and results in formation of cuprous ion (biuret reaction). The Bicinchoninic acid (BCA) reagents reacts with the cuprous ions to form a strong purple colour, with maximum absorbance at 540nm (Assay et al., 2000).

Reduced Glutathione

Tissue glutathione was estimated as described by Laboratorien & Jenapharm (1962). The non-protein compound containing the sulfhydryl group in its structure is known as Glutathione reductase. It reduces 5,5'-dithiobis-2-nitrobenzoic acid to a deep yellow colored compound. The GSH activity was measured by spectrophotometer.

Catalase

Tissue catalase was estimated as described by Aebi (1984). The colon was homogenized and mixed with 50 mmol/L PBS (pH 7.0) and 20 mmol/L hydrogen peroxide. The catalase activity was measured by spectrophotometer.

Superoxide dismutase

Superoxide radicals present in supernatant oxidizes adrenaline bitartarate to form adrenochrome. Estimating the amount of adrenochrome formed is related to SOD activity as it will inhibit oxidation of adrenaline bitartarate by eliminating superoxide radicals (Kuninaka et al., 2000).

Lipid peroxidase by MDA assay

Polyunsaturated fatty acid breaks down to form malondialdehyde, which helps to determine the extent of peroxidation reaction. Pink color is formed by the reaction between thiobarbituric acid and malondialdehyde which is measured at 532nm (Ohkawa et al., 1979).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from colonic tissues using Trizol reagent (Genext Scientific & IT Solutions, Mangalore) referring to the manufacturer's protocol.

Primers (Designed by Genext Scientific & IT Solutions, Mangalore)

TNF α Forward 5' CACCATGAGCACGGAAAGCA 3'

Reverse 5' GCAATGACTCCAAAGTAGACC 3'

GAPDH: Forward 5' CAACTCCCTCAAGATTGTCAGCAA 3'

Reverse 5' GGCATGGACTGTGGTCATGA 3'

Method of RT-PCR

The total RNA was extracted from rat tissue biopsies homogenized in TRI-Reagent (T9424, Merck, India) following the manufacturer's protocol. The extracted RNA was carefully assessed for its quality, purity and integrity using the 260/280 ratio, 260/230 ratio obtained from BioSpectrometer basic, (6135000009, Eppendorf, India) and agarose gel electrophoresis (intact gel bands corresponding to 28S and 18S RNA) respectively. The extracted RNA from each group was diluted to 180 ng/µl using nuclease free water to ensure a known amount of starting mRNA concentration from every group for cDNA synthesis. High-Capacity Reverse Transcriptase cDNA synthesis kit (ThermoFisher Scientific India Pvt. Ltd., India) was used to prepare cDNA (final volume of 20 µl) from the extracted total RNA using thermocycler (T100, BioRad, India). Synthesis of cDNA involved the use of random primers from the cDNA synthesis kit with internal controls as +RT/-RT (with or without reverse transcriptase) to reduce experimental errors and track specificity of primers in case of genomic DNA contamination. .

PCR was performed with a total volume of 25 µL. PCR mixture comprised: cDNA -4 µL, forward and reverse primers - 4 µL each, GoTaq Green master mix -12.5 µL and nuclease free water -4.5 µL. The reaction mixture was then subjected to 33 cycles which consists of 5 minutes pre-apo morphosis at 95°C,

30 seconds apo morphosis at 94°C, 30 seconds annealing at 54.1°C and 1 minute extension at 72°C. Then 10 µL of the ampicon was added to 1.5% agarose gel for electrophoresis. Gel doc (Gel Doc EZ Imager,BioRad, India) was used to capture the image of the gel followed by analysis using the Image- J software (Fiji) (Perera et al., 2010).

Statistical analysis

Statistical analysis was done using Graph Pad Prism 5.03 Demo Version (Graph Pad Software Inc., La Jolla, CA, USA) by one-way analysis of variance (Tukey test). Results were expressed as Mean ± SEM, and $p \leq 0.05$ was considered significant. The relative mRNA expression of TNFα was analyzed by Image-J 1.51f software (Wayne Rasband, National Institutes of Health, USA) using ANOVA (Dunnett's test).

Results

In UC group, the colonic weight **(Figure 1)** was increased compared to the control group. Following seven days of pre-treatment with Sulfasalazine in standard and TFG in test groups, marked reduction in colon weights was observed in them compared to UC- group. There was a significant reduction in DAI, macroscopic and microscopic score in standard and TFG treated groups compared to UC-group. In the UC group, there was intense hyperaemia, ulcerations, and inflammation. In standard and TFG groups, there was a reduction in ulceration and inflammation compared to UC group **(Table 1)**.

Table 1 Effect of TFG on DAI, macroscopic and microscopic changes in rats

Groups	DiseaseActivity Index(DAI)	Macroscopic score	Microscopic score
Normal control	0±0	0±0	0±0
UC	4.00±0.00	4.66±0.21	4.75±0.34
Standard (Sulfasalazine 100mg/kg)	3.33±0.21*	4.00±0.25*	3.50±0.34*
TFG I (500mg/kg)	2.66±0.21*	3.16±0.30*	1.25±0.34*
TFG II (1000mg/kg)	3.50±0.22*	3.83±0.40*	3.25±0.34*

TFG- *Trigonella foenum greacum* L. Values represented as mean ± SEM, n=6 in each group with *$p \leq 0.05$ vs. UC group.

Histopathological changes

In normal Control group, the colon sections had normal architecture with no signs of ulceration and inflammation **(Figure 2A)**. In UC group, there was extensive ulceration and inflammation with a maximal microscopic damage indicating focal necrosis of mucosal and submucosal region with involvement of serosa. Diffuse leukocyte infiltration and crypt damage was observed **(Figure 2B)**. In the groups treated

with sulfasalazine, TFG- I (500mg/kg) and TFG- II (1000mg/kg), the degree of inflammation was lesser compared to UC group and there were no signs of crypt damage or ulceration. **(Figure 2C, 2D, 2E)**.

Biochemical analysis

The total protein level **(Figure 3a)** was decreased (P< 0.05) in UC group. The total protein levels significantly increased in standard, and TFG treated groups compared to the UC group. CAT, SOD and GSH activity **(Figure 3b, 3c and 3d)** was significantly ($P < 0.05$) decreased in colon tissues of UC rats compared to normal control. Pre-treatment with TFG at both doses increased CAT, SOD and GSH activity in colon as compared to UC group. The lipid peroxidase levels decreased significantly in standard, and TFG treated groups compared to the UC group **(Figure 3e)**.

Relative mRNA expression of TNF α

As shown in **Figure 4**, the mRNA levels of TNF α in UC group showed a significantly high expression compared to control group ($p < 0.0001$). The mRNA expressions of TNF α were inhibited in animals treated with Sulfasalazine and TFG (500mg/kg and 1000mg/kg).

Discussion

The present original study has demonstrated the beneficial effect of TFG in UC in rats. The histological changes observed with AA induced colitis in rats is similar to UC seen in humans. AA affects the distal colon and causes inflammation, edema and ulceration of mucosal and submucosal layers. The aqueous extract of TFG has shown protection against colitis in rats, as evidenced by colon length, weight, DAI, gross, histological and biochemical evaluations.

The colon weight/length ratio indicates the intestinal inflammation with the consequent shortening of the colon and increase in weight. There was a significant reduction in the colon weight/length ratio in groups treated with *TFG*, indicating its anti-inflammatory property.

DAI assess disease severity of ulcerative colitis as described by Cooper et al. (1993). It is a combined score of rectal bleeding and stool consistency and was assessed after colitis induction. The DAI score was significantly decreased in groups treated with *TFG* indicating its ability to diminish the intensity of inflammation in ulcerative colitis (Maheshwari et al., 2015).

Previous reports have demonstrated the anti-inflammatory activity of TFG in rat model (Vyas et al., 2008). In the present study, the macroscopic score and microscopic scores which quantitatively measures severity by assessing the depth and extent of ulceration and inflammation in different layers of affected colon, was found to be reduced in TFG treated groups. Similarly, Mandegary et al. (2012) reported the anti-inflammatory effects of TFG and demonstrated that flavonoid component as contributor for the observed beneficial activity. TFG contains phytoconstituents like flavonoids and saponins which could play a critical role in countering the progressive inflammatory challenge in UC (Patil & Jain, 2014).

TNFα is an inflammatory marker and is implicated in the pathogenesis of UC. Studies have shown its production is upregulated in colon tissue in IBD and the levels closely correlate with mayo endoscopic score (severity of UC). Its importance is proven by the robust improvement in symptoms observed in refractory or immunosuppressive intolerant cases receiving anti-TNFα antibody therapy (Hendrickson et al., 2002), (Ślebioda & Kmieć, 2014), (Mańkowska-Wierzbicka et al., 2015). In our experiment, treatment with TFG effectively mitigated TNFα gene expression at mRNA level, which could explain the decreased intensity of colonic inflammatory reaction observed in the group.

Oxidative stress plays an important role in the occurrence and progression of UC (Kruidenier & Verspaget, 2002). The mucosal damage found in IBD is due to increased oxidative stress and decreased antioxidant defense (Koutroubakis et al., 2004). Experimentally induced colitis using acetic acid in animals is due to an imbalance between oxidative stress and antioxidant activity (Bitiren et al., 2010). Oxidative stress is reported to cause mucosal damage in IBD (Rezaie et al., 2007). Rats treated with *TFG* showed protection against lipid peroxidation by demonstrating significant decrease in MDA level and rise in antioxidant levels. Previous report confirms the natural antioxidants ability of flavonoids (Al-Rejaie et al., 2013). The increased levels of antioxidants following TFG could be attributed to the flavonoid and other natural antioxidant components like trigonelline and choline alkaloids (Patil & Jain, 2014). Suppression of oxidative stress and its ability to reduce TNF-α could explain the beneficial effect of TFG against the colon tissue damage, as experiments have demonstrated that these two factors are the triggers for the destructive inflammatory response observed in IBD (Mizushima et al., 2010).

Limitations

- The study did not assess other proinflammatory cytokines apart from TNF-alfa. Also, the TNF-alfa expression was investigated at mRNA level and not the proteins.
- The acetic acid induced colitis may not mimic exactly the inflammatory features of chronic inflammation in humans, though it is an established model for UC in animals.
- Further, molecular studies on inflammatory signalling are required to precisely delineate the protective mechanisms of TFG in UC. However, this study gives first-hand assertion of beneficial effects of TFG in UC model.

Conclusion

Our study confirms the beneficial effects of *Trigonella foenum greacum* L. at both the doses in attenuating the acetic acid induced colitis in a rat model. The study confirms the underlying benefits due to its antioxidant and anti-inflammatory properties. Both the doses of *Trigonella foenum greacum* L. seeds extract were equally effective. The study suggests the potential of *Trigonella foenum greacum* L. seed extract in treating UC patients. However, the clinical benefits needs to be confirmed by further clinical studies.

Declarations

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Compliance with ethical standards

Conflict of interest The authors declare that there were no conflicts of interest.

References

1. Aebi, H. (1984). [13] Catalase in Vitro. *Methods in Enzymology*, *105*(C), 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3
2. Al-Rejaie, S. S., Abuohashish, H. M., Al-Enazi, M. M., Al-Assaf, A. H., Parmar, M. Y., & Ahmed, M. M. (2013). Protective effect of naringenin on acetic acid-induced ulcerative colitis in rats. *World Journal of Gastroenterology*, *19*(34), 5633–5644. https://doi.org/10.3748/wjg.v19.i34.5633
3. Aleisa, A. M., Al-Rejaie, S. S., Abuohashish, H. M., Ola, M. S., Parmar, M. Y., & Ahmed, M. M. (2014). Pretreatment of Gymnema sylvestre revealed the protection against acetic acid-induced ulcerative colitis in rats. *BMC Complementary and Alternative Medicine*, *14*, 1–11. https://doi.org/10.1186/1472-6882-14-49
4. Assay, B., Quantitation, F. O. R., & Total, O. F. (2000). *Ps0304.Pdf*. 1–24.
5. Bitiren, M., Karakilcik, A. Z., Zerin, M., Ozardalı, I., Selek, S., Nazlıgül, Y., Ozgonul, A., Musa, D., & Uzunkoy, A. (2010). Protective effects of selenium and vitamin e combination on experimental colitis in blood plasma and colon of rats. *Biological Trace Element Research*, *136*(1), 87–95. https://doi.org/10.1007/s12011-009-8518-3
6. Button, L. A., Roberts, S. E., Goldacre, M. J., Akbari, A., Rodgers, S. E., & Williams, J. G. (2010). Hospitalized prevalence and 5-year mortality for IBD: Record linkage study. *World Journal of Gastroenterology*, *16*(4), 431–438. https://doi.org/10.3748/wjg.v16.i4.431
7. Cooper, H. S., Murthy, S. N., Shah, R. S., & Sedergran, D. J. (1993). Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Laboratory Investigation; a Journal of Technical Methods and Pathology*, *69*(2), 238—249. http://europepmc.org/abstract/MED/8350599
8. Gálvez, J., Coelho, G., Crespo, M. E., Cruz, T., Rodríguez-Cabezas, M. E., Concha, A., Gonzalez, M., & Zarzuelo, A. (2001). Intestinal anti-inflammatory activity of morin on chronic experimental colitis in the rat. *Alimentary Pharmacology and Therapeutics*, *15*(12), 2027–2039. https://doi.org/10.1046/j.1365-2036.2001.01133.x
9. Hendrickson, B. A., Gokhale, R., & Cho, J. H. (2002). Clinical aspects and pathophysiology of inflammatory bowel disease. *Clinical Microbiology Reviews*, *15*(1), 79–94. https://doi.org/10.1128/CMR.15.1.79-94.2002
10. Joshi, D. V., Patil, R. R., & Naik, S. R. (2015). Hydroalcohol extract of Trigonella foenum-graecum seed attenuates markers of inflammation and oxidative stress while improving exocrine function in

diabetic rats. *Pharmaceutical Biology*, *53*(2), 201–211. https://doi.org/10.3109/13880209.2014.913296

11. Joshi, S. V., Vyas, B. A., Shah, P. D., Shah, D. R., Shah, S. A., & Gandhi, T. R. (2011). Protective effect of aqueous extract of Oroxylum indicum Linn. (root bark) against DNBS-induced colitis in rats. *Indian Journal of Pharmacology*, *43*(6), 656–661. https://doi.org/10.4103/0253-7613.89821
12. Koutroubakis, I. E., Malliaraki, N., Dimoulios, P. D., Karmiris, K., Castanas, E., & Kouroumalis, E. A. (2004). Decreased total and corrected antioxidant capacity in patients with inflammatory bowel disease. *Digestive Diseases and Sciences*, *49*(9), 1433–1437. https://doi.org/10.1023/B:DDAS.0000042242.22898.d9
13. Kruidenier, L., & Verspaget, H. W. (2002). Review article: Oxidative stress as a pathogenic factor in inflammatory bowel disease - Radicals or ridiculous? *Alimentary Pharmacology and Therapeutics*, *16*(12), 1997–2015. https://doi.org/10.1046/j.1365-2036.2002.01378.x
14. Kuninaka, S., Ichinose, Y., Koja, K., & Toh, Y. (2000). Suppression of manganese superoxide dismutase augments sensitivity to radiation, hyperthermia and doxorubicin in colon cancer cell lines by inducing apoptosis. *British Journal of Cancer*, *83*(7), 928–934. https://doi.org/10.1054/bjoc.2000.1367
15. Laboratorien, W., & Jenapharm, V. E. B. (1962). *Zusammen [assung . Deparlment o / Medicine , City o / Hope Medical Center , Duarte (Cali / ornia , U . S . A .), September 3 , 1962 . Values for Serum N u m e r o u s p a p e r s o n lactic d e h y d r o g e n a s e (LDH) activ- C3H] Fg*. *114*(1954), 96–97.
16. Li, Y., Huang, T. H. W., & Yamahara, J. (2008). Salacia root, a unique Ayurvedic medicine, meets multiple targets in diabetes and obesity. *Life Sciences*, *82*(21–22), 1045–1049. https://doi.org/10.1016/j.lfs.2008.03.005
17. Lih-Brody, L., Powell, S. R., Collier, K. P., Reddy, G. M., Cerchia, R., Kahn, E., Weissman, G. S., Katz, S., Floyd, R. A., McKinley, M. J., Fisher, S. E., & Mullin, G. E. (1996). Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. *Digestive Diseases and Sciences*, *41*(10), 2078–2086. https://doi.org/10.1007/BF02093613
18. Maheshwari, R. A., Balaraman, R., Sailor, G. U., & Sen, D. B. (2015). Protective effect of simvastatin and rosuvastatin on trinitrobenzene sulfonic acid-induced colitis in rats. *Indian Journal of Pharmacology*, *47*(1), 17–21. https://doi.org/10.4103/0253-7613.150311
19. Mandegary, A., Pournamdari, M., Sharififar, F., Pournourmohammadi, S., Fardiar, R., & Shooli, S. (2012). Alkaloid and flavonoid rich fractions of fenugreek seeds (Trigonella foenum-graecum L.) with antinociceptive and anti-inflammatory effects. *Food and Chemical Toxicology*, *50*(7), 2503–2507. https://doi.org/10.1016/j.fct.2012.04.020
20. Mańkowska-Wierzbicka, D., Swora-Cwynar, E., Poniedziałek, B., Adamski, Z., Dobrowolska, A., & Karczewski, J. (2015). Usefulness of selected laboratory markers in ulcerative colitis. *European Cytokine Network*, *26*(2), 26–37. https://doi.org/10.1684/ecn.2015.0363
21. Mizushima, T., Sasaki, M., Ando, T., Wada, T., Tanaka, M., Okamoto, Y., Ebi, M., Hirata, Y., Murakami, K., Mizoshita, T., Shimura, T., Kubota, E., Ogasawara, N., Tanida, S., Kataoka, H., Kamiya, T., Alexander, J.

S., & Joh, T. (2010). Blockage of angiotensin II type 1 receptor regulates TNF-α-induced MAdCAM-1 expression via inhibition of NF-κB translocation to the nucleus and ameliorates colitis. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, *298*(2). https://doi.org/10.1152/ajpgi.00264.2009

22. 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*(2), 795–803. https://doi.org/10.1016/S0016-5085(89)80079-4
23. Muralidhara, Narasimhamurthy, K., Viswanatha, S., & Ramesh, B. S. (1999). Acute and subchronic toxicity assessment of debitterized fenugreek powder in the mouse and rat. *Food and Chemical Toxicology*, *37*(8), 831–838. https://doi.org/10.1016/S0278-6915(99)00076-9
24. Noor, E. A. (2007). Temperature effects on the corrosion inhibition of mild steel in acidic solutions by aqueous extract of fenugreek leaves. *International Journal of Electrochemical Science*, *2*(12), 996–1017. https://doi.org/10.3923/jeasci.2008.23.30
25. 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
26. Parkes, M., & Jewell, D. (2001). Ulcerative colitis and Crohn's disease: Molecular genetics and clinical implications. *Expert Reviews in Molecular Medicine*, *3*(30), 1–18. https://doi.org/10.1017/S146239940100391X
27. Patil, S., & Jain, G. (2014). Holistic approach of Trigonella foenum-graecum in Phytochemistry and Pharmacology- A Review. *Current Trends in Technology and Science*, *3*(1), 34–48.
28. Perera, P. K., Li, Y., Peng, C., Fang, W., & Han, C. (2010). Immunomodulatory activity of a Chinese herbal drug Yi Shen Juan Bi in adjuvant arthritis. *Indian Journal of Pharmacology*, *42*(2), 65–69. https://doi.org/10.4103/0253-7613.64489
29. Rezaie, A., Parker, R. D., & Abdollahi, M. (2007). Oxidative stress and pathogenesis of inflammatory bowel disease: An epiphenomenon or the cause? *Digestive Diseases and Sciences*, *52*(9), 2015–2021. https://doi.org/10.1007/s10620-006-9622-2
30. Sartor, R. B. (1997). Pathogenesis and immune mechanisms of chronic inflammatory bowel diseases. *The American Journal of Gastroenterology*, *92*(12 Suppl), 5S-11S.
31. Ślebioda, T. J., & Kmieć, Z. (2014). Tumour necrosis factor superfamily members in the pathogenesis of inflammatory bowel disease. *Mediators of Inflammation*, *2014*. https://doi.org/10.1155/2014/325129
32. Vyas, S., Agrawal, R. P., Solanki, P., & Trivedi, P. (2008). Analgesic and anti-inflammatory activities of Trigonella foenum-graecum (seed) extract. *Acta Poloniae Pharmaceutica - Drug Research*, *65*(4), 473–476.
33. Yadav, U. C. S., & Baquer, N. Z. (2014). Pharmacological effects of Trigonella foenum-graecum L. in health and disease. *Pharmaceutical Biology*, *52*(2), 243–254. https://doi.org/10.3109/13880209.2013.826247

Figures

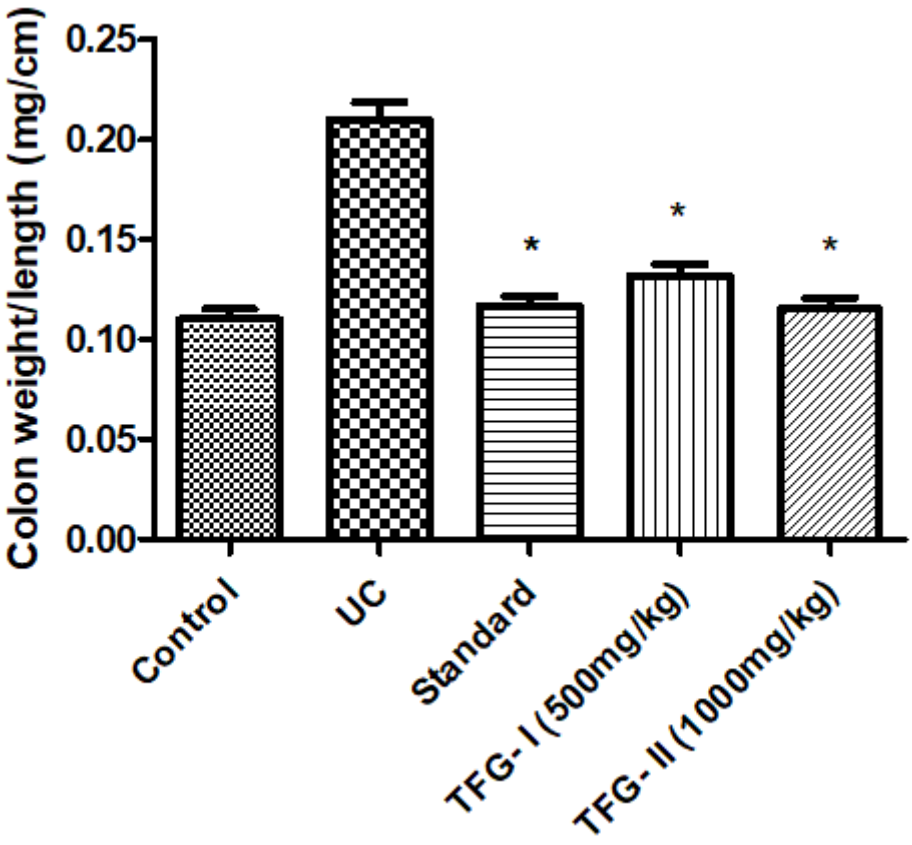

Figure 1

Effect of TFG on colon weight/length in rats. Data represented as mean ± SEM and analysed using with * $p \leq 0.05$ vs. UC group.

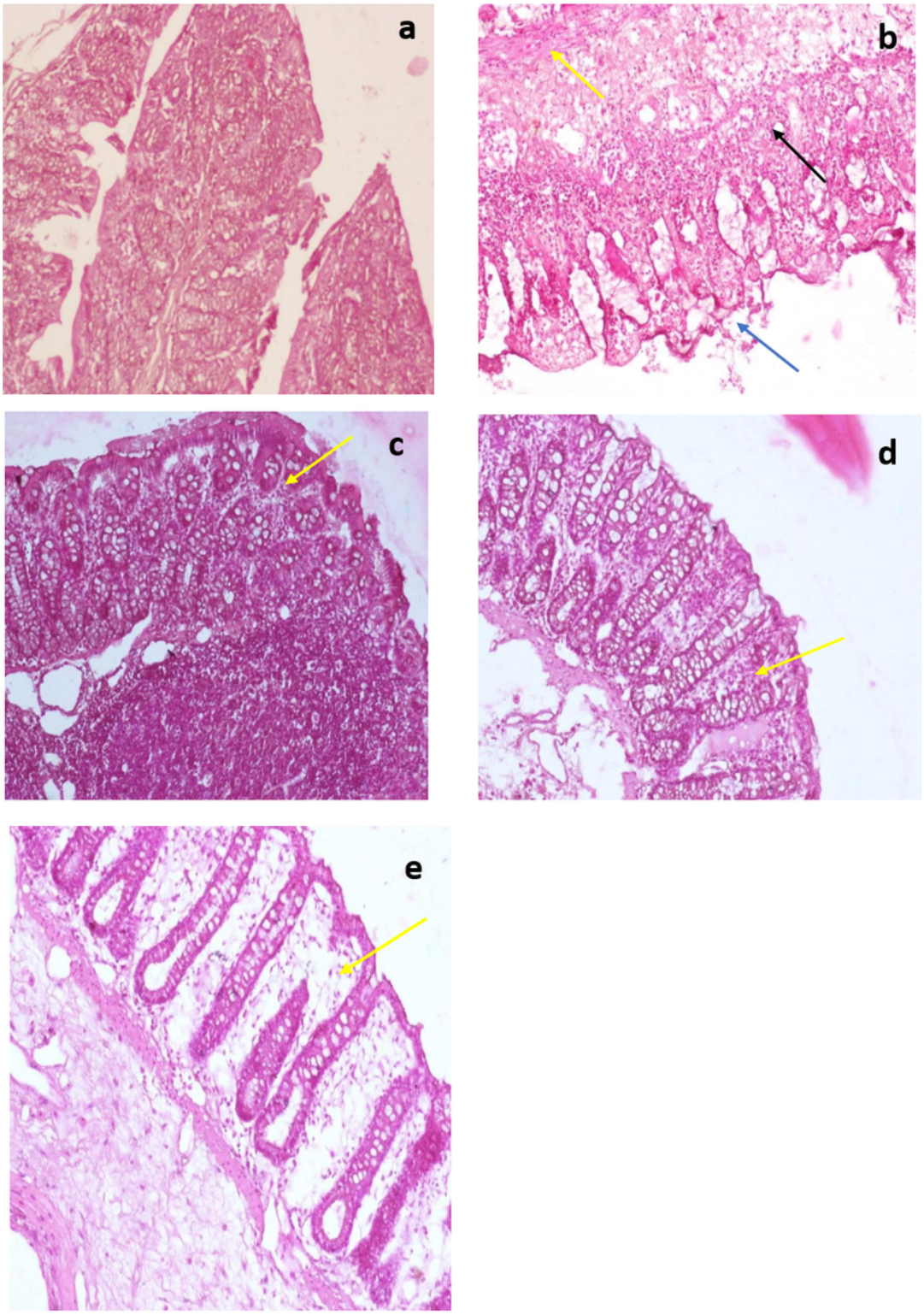

Figure 2

Effect of TFG on histological features in rats. a: Control, b: UC- group, c: Standard, d: TFG- I (500mg/kg), e: TFG- II (1000mg/kg) BLACK ARROW: Ulceration YELLOW ARROW: Transmural inflammation BLUE ARROW: Crypt damage

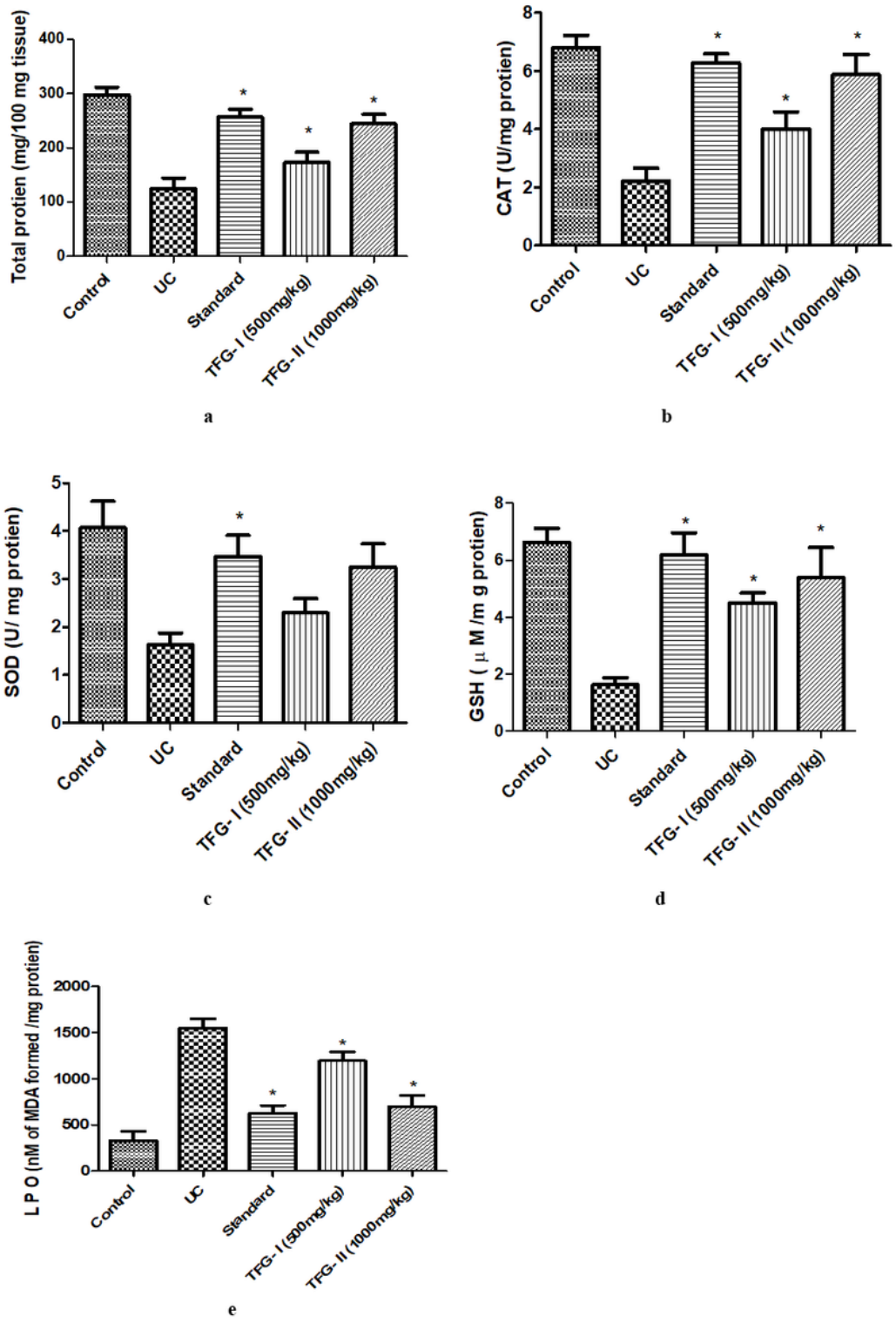

Figure 3

Effect of TFG on biochemical parameters [a] Total protein [b] CAT [c] SOD [d] GSH and [e] LPO. Data represented as mean ± SEM and analysed using ANOVA with * $p \leq 0.05$ vs. UC group.

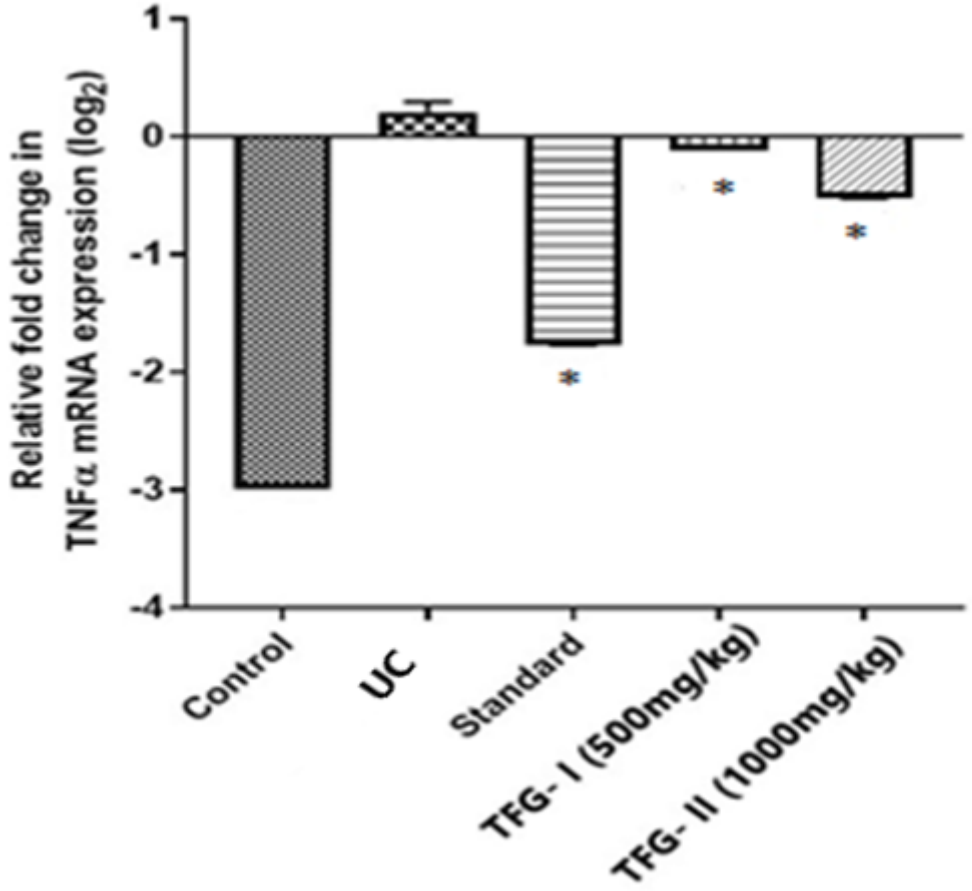

Figure 4

Effect of TFG on Relative mRNA expression of TNFα in rats. Data represented as mean ± SEM and analysed using ANOVA followed by Dunnett's analysis with *p≤0.001 vs. UC group.