

## Ulcerative Colitis-induced Hematotoxicity in Mice and its Amelioration with Visnagin

V. Sravathi, D. Madhuri\*, Y. Ravi Kumar, B. Anil Kumar, A. Vijaya Kumar, B. Swathi and S. Soujanya

Department of Veterinary Pathology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana-500030

\*Email: madhurighanta@yahoo.co.in

Received: July 2024

141/24

Accepted: December 2024

### ABSTRACT

Ulcerative Colitis (UC) is the most challenging disease in humans and animals. The present study was done to explore the potential effect of Visnagin against Dextran Sodium Sulphate (DSS) induced UC in mice, and its potential mechanism. Visnagin, a natural compound, has a range of antioxidant and anti-inflammatory effects. The mice were euthanized on the 32<sup>nd</sup> day of the experimental period, and the haematological parameters were assessed to evaluate the efficacy of the treatment. The results indicated significant improvements in the haematological parameters, suggesting that visnagin can effectively mitigate the haematological impacts of DSS-induced ulcerative colitis in mice.

**Keywords:** Mice, Dextran sodium sulphate, Ulcerative colitis, Dexamethasone, Visnagin

### INTRODUCTION

Ulcerative Colitis (UC) is a common form of inflammatory bowel disease, characterized by chronic inflammation and ulcers in the colon and rectum, leading to recurring phases of exacerbation and remission in dogs and cats (Abou Zaid *et al.*, 2023). Additionally, UC can cause numerous complications and symptoms, such as intermittent diarrhoea, constipation, cramping, abdominal and rectal pain, bleeding and anaemia. The common cause of diarrhoea in animals is chronic intestinal disease with lesions appearing similar to those of humans (Panah *et al.*, 2021). The current treatment approaches include the use of anti-inflammatory drugs (such as 5-aminosalicylates and corticosteroids), immunosuppressants

(Thiopurines and methotrexate), immunomodulators (anti-TNF antibodies) and surgery in cases of complications (Meineri *et al.*, 2022). However, these medications are often associated with adverse side effects that limit their long-term viability. A potential new therapy for these conditions involves the use of antioxidants through natural products. Natural byproducts are regarded as a promising source of effective and safe treatments for these diseases. Visnagin, obtained from the extract of *Ammivisnaga*, has potent pharmacological effects. Visnagin is used to treat urolithiasis and angina pectoris and acts as a vasodilating agent. It modulates various organ toxicities due to its anti-inflammatory, antioxidant and immunosuppressive effects.

### MATERIALS AND METHODS

A total of 36 male C57BL/6 mice (25-30 g) were procured from Jeeva Life Sciences, Hyderabad. The experimental protocol received approval from the Institutional Animal Ethics Committee (IAEC) under approval no. 06/26/C.V.Sc, Hyd. IAEC. Mice were divided into six groups (n=6): group1 (sham), group 2 (DSS) model @ 2% diluted in drinking water with 5 days interval for 31 days, group-3 (visnagin @ 60mg/kg BW), groups-4 and 5 low and high dose visnagin + DSS @ 30 and 60 mg/kg BW, respectively and Group-6 treated with dexamethasone @ 1 mg/kg BW and DSS, given simultaneously with DSS in groups 3, 4, 5 and 6 for 31 days. All mice were sacrificed at the end of the experiment period (32<sup>nd</sup> day) after fasting overnight. Approximately 0.5 ml of blood was collected from the retro-orbital plexus through capillary

tubes into EDTA vacutainers on the 32<sup>nd</sup> day of the experiment for haematological investigation. All blood samples were analysed using an automatic whole blood analyzer (Huma count, Med Source Ozone Biomedical Pvt. Ltd., Faridabad, Haryana). Results were tabulated for statistical analysis using GraphPad Prism version 9.0 software (GraphPad Software, California, USA), followed by Tukey's multiple comparison test. A P-value of less than 0.001 was considered statistically significant.

## RESULTS AND DISCUSSION

Changes in haematological parameters due to tissue damage were used to ascertain the severity of colitis. Induction of colitis with DSS was confirmed by the symptoms of bloody diarrhoea, resulting in significantly ( $P<0.001$ ) lowered TEC, Hb and PCV, while significantly ( $P<0.001$ ) higher TLC and neutrophil counts were noted in group 2 compared to group 1. This may be attributed to chronic inflammation-induced anaemia and severe gastrointestinal bleeding caused by DSS. Anaemia is the most common extraintestinal complication in UC (Shah *et al.*, 2021). Iron and vitamin B<sub>12</sub> deficiency due to blood loss, impaired uptake, reduced absorption time, and low levels of serum protein/albumin are the major reasons for anaemia in UC. Chronic inflammation interferes with erythropoiesis and iron metabolism, while mucosal ulceration leads to blood loss resulting in decreased RBC, Hb and PCV levels. Additionally, there was a significant increase in TLC count, especially neutrophils, due to the chronic inflammatory effect, which increases T cell activity at the inflamed site, providing an initial line of defence for the innate immune system (Mack *et al.*, 2020) (Fig. 1).

Groups 4 and 5 of visnagin-treated mice and the standard group 6 mice showed significant ( $P<0.05$ ,  $P<0.001$  and  $P<0.001$ , respectively) improvement in these mean values compared

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to group 2. Notably, group 5 mice showed a significant ( $P<0.05$ ) improvement compared to group 4 in a dose-dependent manner. However, there were no significant differences observed between groups 5 and 6 except in TLC, while no significant difference was noted in the mean values of group 1 and group 3, indicating the safety of visnagin compound. This might be due to the ameliorative effects of VIS, which restore haematological parameters through its anti-inflammatory and anti-oxidant properties (Pasari *et al.*, 2019). A previous study demonstrated that visnagin effectively protected against cerulenin-induced pancreatitis in animals by increasing Nrf2 expression and decreasing NF- $\kappa$ B levels (Pasari *et al.*, 2019). Additionally, visnagin showed anti-inflammatory properties by reducing NF- $\kappa$ B expression in lipopolysaccharide-stimulated microglial cells. Asnani *et al.* (2018) reported that visnagin exhibited cardioprotective effects against doxorubicin-induced cardiomyopathy. Additionally, dexamethasone, a corticosteroid, acts by regulating the synthesis of proteins. This regulation inhibits the migration of leukocytes to sites of tissue damage and phagocytosis, along with reducing permeability of capillaries, thus exerting an anti-inflammatory effect and preventing hematotoxicity in group 6 (Zhdanova *et al.*, 2024).

## CONCLUSIONS

In this experimental study, visnagin at different doses (30 and 60 mg/kg BW) was evaluated against the DSS-induced UC in mice and compared with the standard drug dexamethasone at 1 mg/kg BW. Both doses significantly ameliorated haematological parameters against DSS-induced UC through their strong anti-inflammatory and anti-oxidant properties, proving that it is an effective therapeutic agent for managing UC. Future efforts should focus on elucidating the molecular mechanisms and conducting clinical

trials to confirm the safety and efficacy of this treatment.

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**Figure 1: Ameliorative Effect of Visnagin in DSS-induced Ulcerative Colitis in Mice**



