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RESEARCH ARTICLE

Ameliorative effect of visnagin against colitis derived hepatoxicity by dextran sodium sulphate in C57BL/6 mice

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ABSTRACT

The present study was conducted to investigate hepatic damage associated with Dextran sodium sulphate (DSS) induced ulcerative colitis (UC) and to evaluate the ameliorative effect of Visnagin (VIS) against this hepatic damage. Six groups of C57BL/6 male mice, each containing six animals *viz.* Group 1 (Normal), Group 2 (DSS @ 2% with 3 cycles of 5 day intervals diluted in distilled water administered orally), Group 3 (VIS @ 60 mg/kg b.wt., orally), Group 4 (VIS @ 30 mg/kg b.wt. and DSS), Group 5 (VIS @ 60 mg/kg b.wt. and DSS) and Group 6 (Dexamethasone sodium @ 1 mg/kg b.wt. IP and DSS). After the 31-day experimental period, organ weights, serum analysis, oxidative stress parameters, inflammatory cytokines, gross and histopathology and immunohistochemical analysis were evaluated on day 32. Group 2 mice (DSS only) showed significant increase in ALT, AST, MDA and nitrite levels, along with elevated pro-inflammatory cytokines levels. Conversely, significant decreases in total protein, albumin levels, antioxidant enzymes levels (SOD, CAT and GSH) and IL-10 concentrations were observed. Histopathological examination revealed severe degenerative changes in the livers of Group 2, with increased NF-κB immunoexpression and reduced Nrf2 protein expression. Groups 4 and 5 treated with VIS, showed dose-dependent improvements with moderate to mild changes attributed to VIS's anti-inflammatory and antioxidant properties likely through modulation of the Nrf2 signaling pathway. Group 6 treated with Dexamethasone, also showed improvement in all these parameters. In conclusion, VIS demonstrated notable anti-inflammatory and anti-oxidant properties against DSS-induced hepatotoxicity.

Keywords: Dextran sodium sulphate, hepatic damage, ulcerative colitis, visnagin

INTRODUCTION

The gut-liver axis refers to the bidirectional relationship between the gastrointestinal tract and the liver, and it plays a crucial role in inflammatory bowel disease (IBD)[1]. Ulcerative colitis (UC), a subtype of IBD, is a chronic condition marked by persistent inflammation primarily affecting the colon's mucosal lining. This inflammation disrupts the integrity of the colon mucosa impairing the intestinal barrier function and leading to a range of gastrointestinal symptoms and complications[2]. In addition to the chronic, non-specific intestinal inflammation, IBD often presents with various extra intestinal manifestations which significantly contribute to the morbidity and mortality of affected patients. Among these extra intestinal manifestations, hepatobiliary disorders are relatively common, further complicating the clinical management of IBD[3]. Intestinal inflammation and microbial dysbiosis contribute to liver injury through excessive exposure to bacterial translocation, enteric antigens, toxins and inflammatory mediators which subsequently activate inflammatory signaling pathways such as the toll like receptors/nuclear factor kappa-light-chain-enhancer of activated B cell (TLR4/NF-κB) pathway. This process enhances the secretion of pro-inflammatory cytokines like tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6, thereby exacerbating liver damage and ultimately leading to chronic liver diseases[4]. The inflammatory cascade triggered by DSS results in liver damage characterized by hepatocyte apoptosis, fibrosis and altered liver function. Additionally, medications used to manage IBD can cause gastrointestinal and liver-related side effects[5]. Therefore, targeting inflammatory signaling pathways and regulation of the gut-liver axis present promising strategies for developing drugs to treat liver injury.

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In response to emerging health risks, there is increasing interest in natural products for preventive and therapeutic measures. Visnagin {4-methoxy-7-methylfuro (3,2-g) chromen-5-one} is a funarochrome active compound extracted from the plant of *Ammi visnaga* has demonstrated widespread pharmacological actions including anti-cancer, anti-inflammatory, anti-oxidant, anti-microbial and other biological effects[6]. Previous studies indicate

that VIS prevents damage to renal epithelial cells through its diuretic action. Additionally, VIS has been reported to exhibit cardioprotective and vasodilatory effects[6,7] and to provide efficacy against hypertriglyceridemia, urolithiasis, reducing apoptosis in the follicular tissues of rat ovaries and diminishing inflammatory and oxidative responses in LPS-activated BV-2 microglial cells[8]. Pasari *et al.*[9] recently reported the role of Nrf2 in mediating the anti-inflammatory and antioxidant activities of VIS using a cerulein-induced acute pancreatitis model in mice. Their findings showed that VIS decreased the expression of pro-inflammatory cytokines in a dose-dependent manner. Notably, VIS enhanced antioxidant defenses by upregulating Nrf2 and reduced pancreatic inflammation by suppressing NF-κB expression in acinar cells. Furthermore, VIS inhibited the release of inflammatory cytokines in both pulmonary and intestinal tissues[9]. However, the effects of VIS on liver injury have not yet been analyzed. Therefore, in this study, we aimed to investigate the therapeutic potential of VIS against colitis induced liver injury.

Table 1. Ameliorative effect of VIS on serum biochemical parameters.

Groups	ALT (IU/L)	AST (IU/L)	TP (g/dL)	Albumin (g/dL)
Group 1	53.59±0.42	58.86±3.44	7.12±0.17	3.50±0.11
Group 2	84.60±0.50$^{###}$	235.60±6.15$^{###}$	3.76±0.05$^{###}$	1.60±0.13$^{###}$
Group 3	53.37±0.91	59.59±2.70	7.23±0.07	3.55±0.11
Group 4	78.17±0.40***	212.90±2.80***	4.67±0.12***	2.25±0.10**
Group 5	73.31±0.60***	194.80±1.07***	5.72±0.08***	2.53±0.10***
Group 6	64.98±0.50***	165.90±1.42***	6.51±0.08***	2.90±0.13***

MATERIAL AND METHODS

Reagents

Dextran sodium sulphate salt 500 was procured from Savvy Scientifics (CAT No. 99629) and Visnagin from Cayman Chemical Company, USA (CAT No. 34140). Kits for measuring Aspartate Transaminase (AST), Alanine Transaminase (ALT), total protein (TP) and albumin were purchased from Erba Diagnostics, USA. ELISA kits for TNF-α, TGF-β, IL-1β, IL-6, NF-κB, IL-17 and LI-10 were acquired from Invitrogen, Thermo Fischer Scientific Ltd. Stains and chemicals for the histopathological study of tissues were obtained from HiMEDIA Laboratories Pvt. Ltd., Secunderabad, Telangana, Antibodies for NF-κB, Nrf2, COX-2 and caspase-3 used in immunohistochemical staining were sourced from M/s Santacruz Biotechnology, USA. The PolyExcel HRP/DAB detection system for

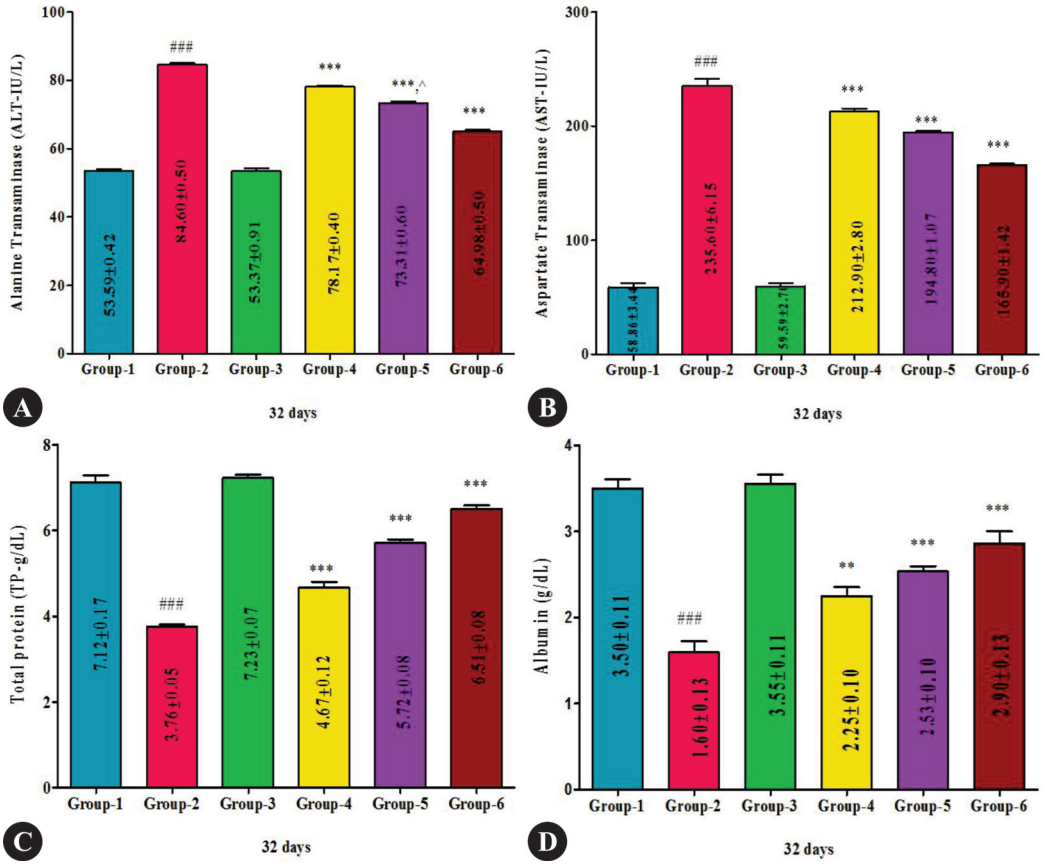

Fig. 1. Ameliorative effect of VIS on serum biochemical parameters. **A.** ALT. **B.** AST. **C.** Total Protein. **D.** Albumin.

Table 2. Effect of visnagin on Oxidative and antioxidant indices in liver of different groups of mice.

Groups	TBARS (nM/mg protein)	Nitrite (µm/mg protein)	SOD (IU/mg protein)	GSH (µM/mg protein)	Catalase (IU/mg protein)
Group 1	15.44±2.17	10.03±0.41	29.75±0.86	47.76±4.30	15.03±0.70
Group 2	59.14±2.64$^{###}$	46.00±1.49$^{###}$	9.45±0.71$^{###}$	14.17±0.83$^{###}$	4.31±0.31$^{###}$
Group 3	16.88±1.25	11.60±0.30	32.16±1.99	45.30±1.90	14.52±0.61
Group 4	33.07±1.22*	25.41±0.44***	14.89±0.33*	25.17±0.80**	7.91±0.40*
Group 5	22.76±1.85***	15.90±0.84***	22.88±1.19***	34.86±1.30***	9.87±0.80***
Group 6	20.66±1.21***	13.04±0.32***	25.14±0.61***	38.38±1.07***	10.25±1.04***

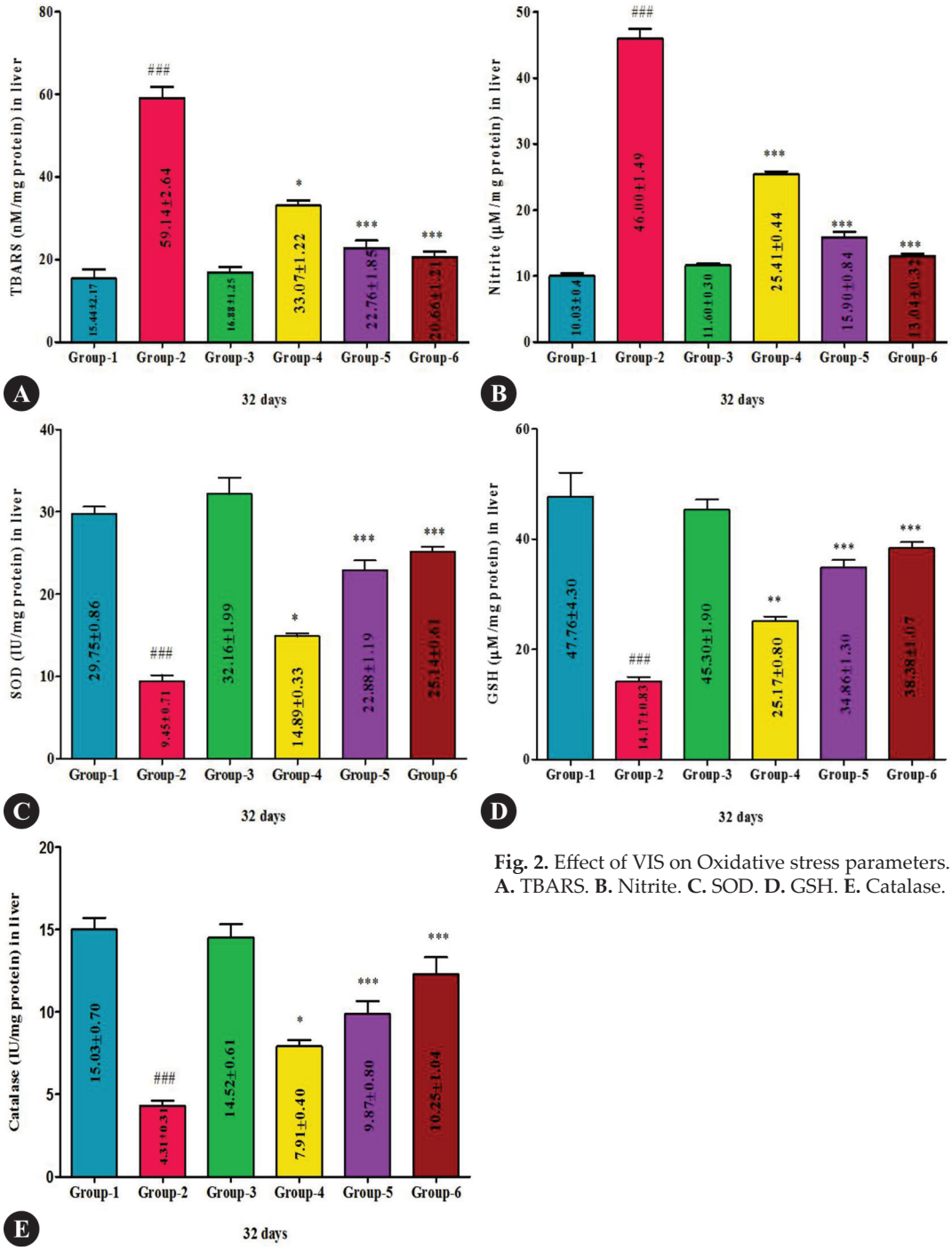

Fig. 2. Effect of VIS on Oxidative stress parameters. **A.** TBARS. **B.** Nitrite. **C.** SOD. **D.** GSH. **E.** Catalase.

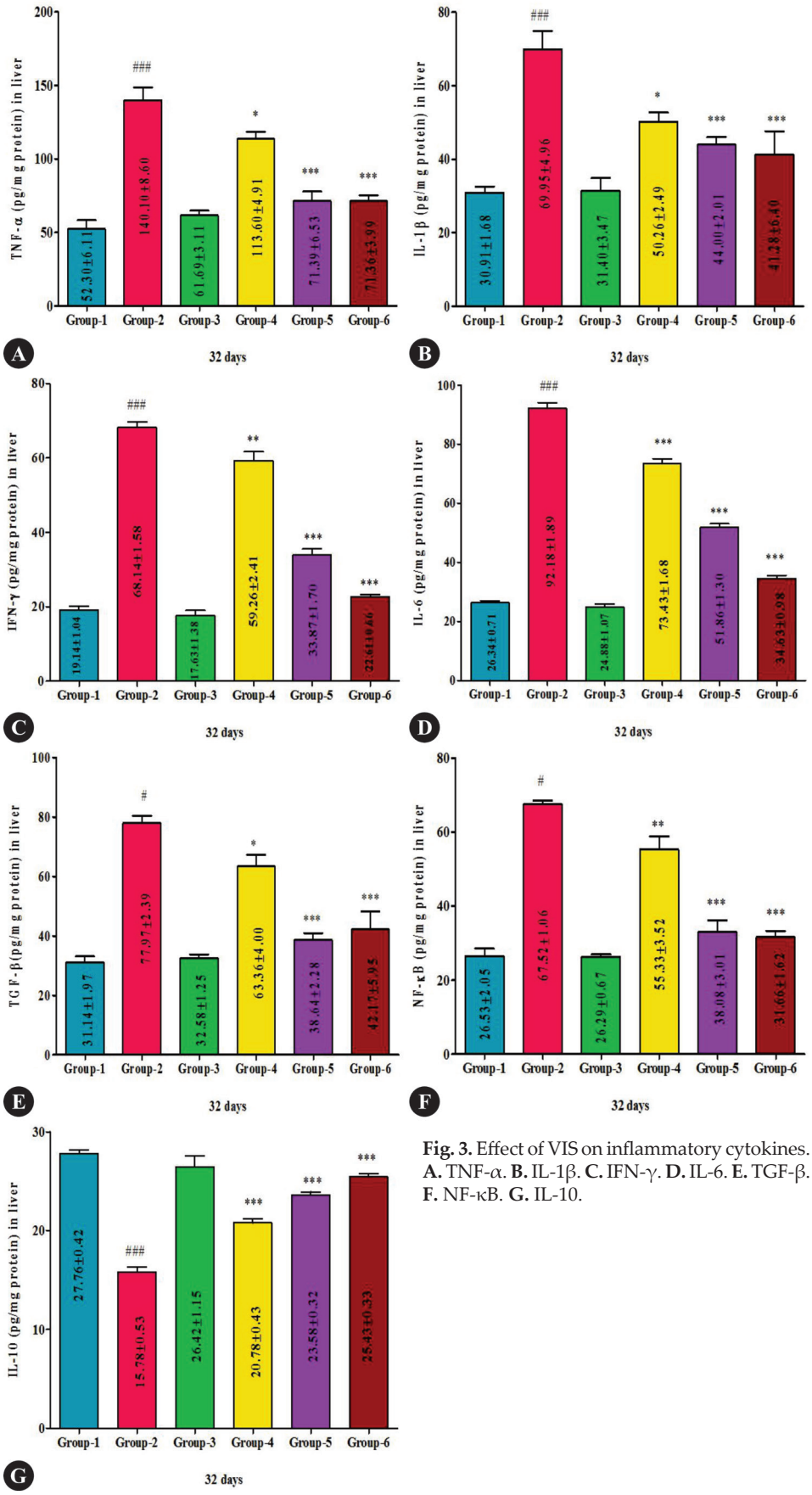

Fig. 3. Effect of VIS on inflammatory cytokines. **A.** TNF-α. **B.** IL-1β. **C.** IFN-γ. **D.** IL-6. **E.** TGF-β. **F.** NF-κB. **G.** IL-10.

Table 3. Effect of visnagin in pro-inflammatory and anti-inflammatory cytokine indices in liver of different groups of mice.

Groups	TNF-α (pg/mg protein)	IL-1β (pg/mg protein)	IFN-γ (pg/mg protein)	IL-6 (pg/mg protein)	IL-10 (pg/mg protein)
Group 1	52.30±6.11	30.91±1.68	19.14±1.04	26.34±0.71	27.76±0.42
Group 2	140.10±8.60$^{###}$	69.95±4.96$^{###}$	68.14±1.58$^{###}$	92.18±1.89$^{###}$	15.78±0.53$^{###}$
Group 3	61.69±3.11	31.40±3.47	17.63±1.38	24.88±1.07	26.42±1.15
Group 4	113.60±4.91*	50.26±2.49*	59.26±2.41**	73.43±1.68***	20.78±0.43***
Group 5	71.39±6.53***	44.00±2.01***	33.87±1.70***	51.86±1.30***	23.58±0.32***
Group 6	71.36±3.99***	41.28±6.40***	22.61±0.66***	34.63±0.98***	25.43±0.33***

immunohistochemistry was procured from M/s PathnSitu Biotechnologies, USA.

Study design

36 male C57BL/6 mice (25-30 gms) were obtained from the M/S Jeeva life sciences Ltd. Hyderabad and were maintained in a controlled environment throughout the course of the experiment. Mice were provided with 7 days of acclimation period before the experiment began and allowed free access to sterile food and drinking water. The experimental protocol was reviewed and approved by Institutional Animal Ethics Committee, CVSc, Rajendranagar, Hyderabad (06/26/CVSc, Hyd. IAEC /2023). To assess the ameliorative effects of visnagin on liver injury caused by DSS induced colitis, the mice were randomly assigned to six experimental groups (n=6). The group 1 (sham control), group 2 (2% DSS with 3 cycles of 5 days' intervals, diluted in distilled water administered orally, group 3 *perse* (visnagin at 60 mg/kg b.wt), groups 4 and 5 (DSS + visnagin at 30 and 60 mg/kg b.wt respectively) and group 6 (standard drug, dexamethasone at 1 mg/kg b.wt) for 31 days. All mice were sacrificed on the 32nd day of the experimental period and liver samples were collected. A portion of liver tissue was preserved in 10% neutral buffer formalin (NBF) for histopathological examination while other portion was stored at -80^{O}C for further analysis.

Biochemical analysis

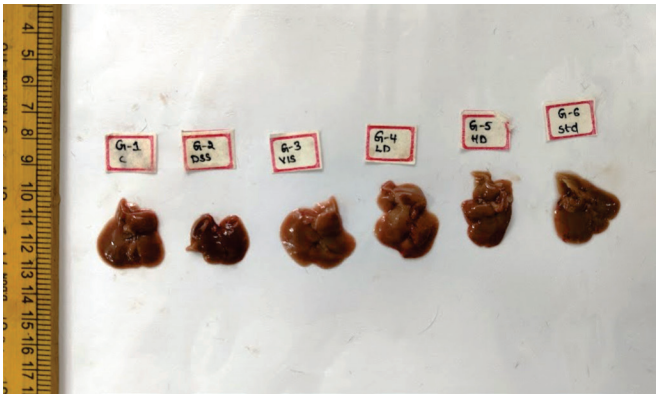

Fig. 4. Severe congestion of the liver observed in group 2 compared to other groups.

Approximately 0.5 mL of blood was collected from the retro-orbital plexus using a capillary tube and transferred into a plain serum vacutainer, allowing it to clot for 3-4 h. The blood samples were then centrifuged at 12,000 rpm for 15 min, serum was separated and stored at -20^{O}C. The serum samples were subsequently used to evaluate biochemical parameters (ALT, AST, TP & albumin) using Erba Mannheim biochemical kits following manufacturer's instructions on a semi-automatic ELISA reader.

Tissue antioxidant profile

Small pieces of liver tissue were collected and stored at -80^{O}C to analyze the organ's antioxidant profiles (GSH, SOD and Catalase) along with oxidative stress levels (TBARS and nitrite). For each gram of tissue sample, 10 mL of 0.2 M Tris HCl buffer was added and homogenized to make a 10% tissue homogenate for assessing oxidative stress parameters.

The tissue oxidation was measured by the reaction of the lipid peroxidation (LPO) end products like Malondialdehyde (MDA), with thiobarbituric acid (TBA) using a standard protocol[10]. Nitrite levels were measured according to the Griess Reagent Kit (Thermo Fisher Scientific Pvt. Ltd.). The activity of superoxide dismutase (SOD)[11], reduced glutathione (GSH)[12] and catalase[11] was measured to determine the antioxidant status of the liver tissue.

Inflammatory cytokine assays

The expression of pro-inflammatory (TNF-α, TGF-β, IL-1β, IL-6, NF-κB, IL-17) and anti-inflammatory (IL-10) cytokines in liver homogenate were measured by sandwich ELISA. The kits were procured from Invitrogen, Thermo Fischer Scientific Ltd. and the assay was performed following the protocol described by Sangomla *et al.* (2018).

Gross, histopathological and scanning electron microscopic examination

Experimental mice were sacrificed by cervical dislocation and detailed necropsy was carried out. Gross lesions if any were recorded in the liver and small slices of liver tissue were collected for histopathological

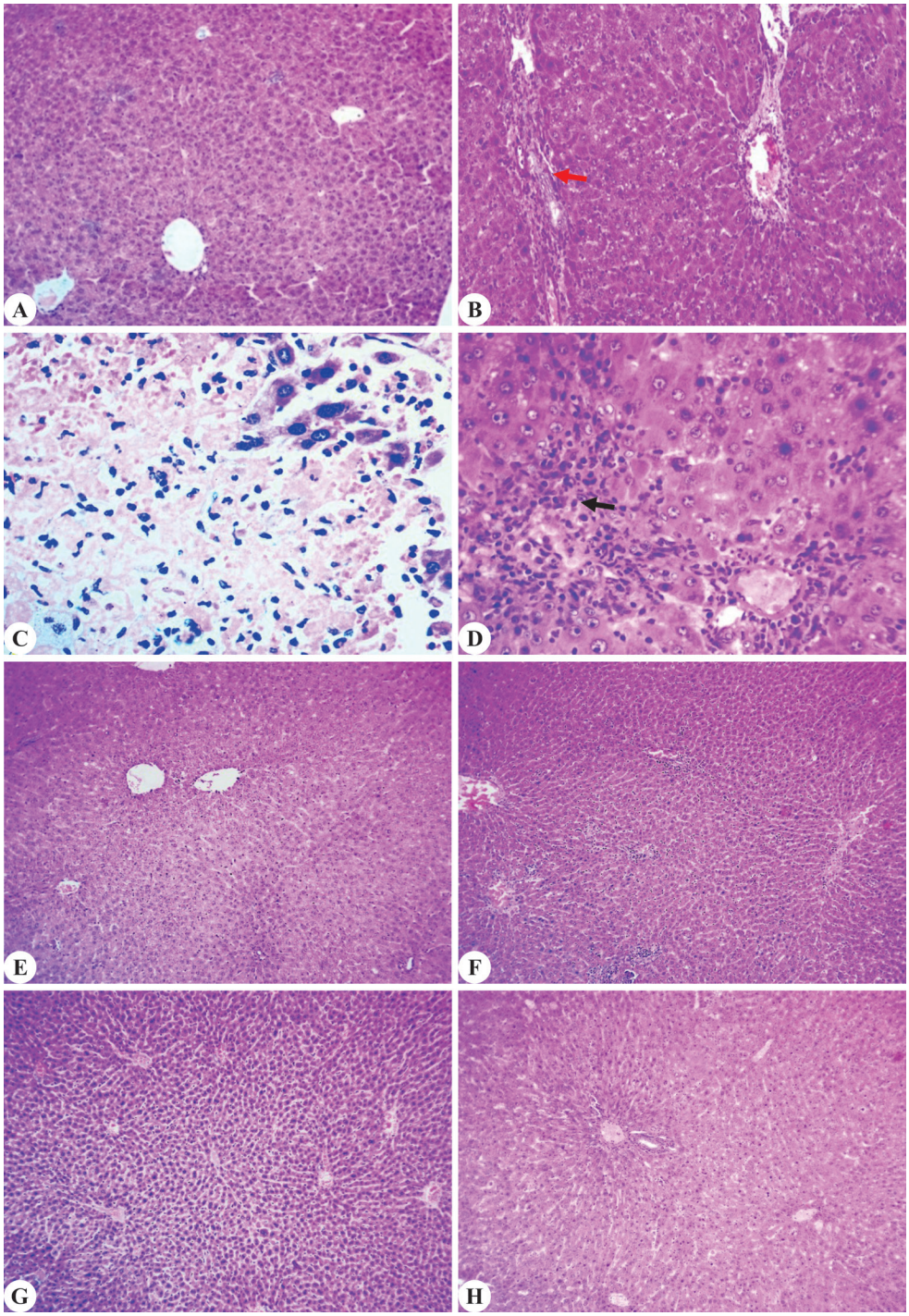

Fig. 5. Effect of VIS on histopathological changes. **A.** Control mice (G-1) Normal hepatic cord arrangement in the hepatic parenchyma (H&E X10). **B.** Severe proliferation of fibroblastic cells (H&E X10). **C, D.** Necrosis and haemorrhages in hepatic parenchyma with severe lymphocytic infiltration in DSS treated group (G-2) (H&E X40). **E.** Normal hepatic cord arrangement in the hepatic parenchyma in ameliorative group (G-3) (H&E X10). **F.** Low dose (G-4). **G.** High dose (G-5). **H.** Standard drug (G-6) moderate to mild changes from group 4 to 6 (H&E X10).

examination in 10% NBF. The samples were processed, sectioned (4 µm) and stained with hematoxylin and eosin as per the standard procedure[13].

Immunohistochemistry

Immunohistochemical analysis was carried out on formalin-fixed, paraffin-embedded sections of liver tissue with a thickness of 4 µm. Briefly, the tissue sections were incubated overnight at 4^{O}C with primary antibodies targeting NF-κB. After incubation, the sections were rinsed with phosphate-buffered saline (PBS) and treated

with a secondary antibody. They were then treated with Diaminobenzidine (DAB) and counter stained with hematoxylin and observed under optical microscope at 100x magnification[14].

Statistical analysis and its interpretation

The findings from current experiments are reported as mean ± SE values. Stastical analysis was performed using graph Pad Prism version 9.0 software (GraphPad Software, California, USA), which exposed to a one-way analysis of variance (ANOVA) applying Tukey's multiple comparison test. A P-value of less than 0.001 was considered statistically significant in each and every group[15].

RESULTS

Ameliorative effect of VIS on serum biochemical parameters

Significantly (P<0.001) higher mean values of serum ALT and AST levels were recorded in the UC-induced group 2 compared to control group 1. Conversely, serum ALT and AST levels were significantly (P<0.01) ameliorated in VIS-treated groups 4 and 5, as well as standard-treated group 6, compared to group 2. Additionally, the values of ALT and AST in group 5 were significantly (P<0.05) lower than those in group 4. Moreover, a significant (P<0.001) decrease in serum TP and albumin concentrations was observed in group 2 compared to normal group 1. However, albumin levels significantly (P<0.01, P<0.001 and P<0.001, respectively) increased in groups 4, 5 and 6 compared to group 2. No significant difference was noticed between groups 5 and 6 as well as between control group 1 and 3 (Fig. 1A-D).

Ameliorative effect of VIS on oxidative stress parameters

Superoxide free radicals primarily released by nitrite and MDA, contribute significantly to oxidative stress. DSS administration caused a significant (P<0.001) rise in highly reactive MDA and nitrite mean values compared to normal group 1. However, treatment groups 4, 5 and the standard group 6 showed a significant (P<0.001) decrease in liver TBARS mean levels compared to group 2. Additionally, group 5 (high dose) displayed significantly (P<0.05) decrease in TBARS levels compared to group 4 (low dose) and group 5 mean values were insignificantly different from group 6. There were no significant variations between group 1 and group 3 (Fig. 2A, B).

The activity of antioxidant enzymes of SOD, CAT and GSH in DSS-treated group 2 were significantly (P<0.001) reduced as compared to the control group 1. In contrast, VIS-treated groups 4, 5 and the standard treated group 6 revealed a significant (P<0.001) increase compared to group 2. Group 5 showed significantly (P<0.05) greater improvement than group 4 in a dose-dependent manner. Additionally, SOD and CAT levels in mice from group 5 were comparable to those in group 6. No significant variation was found in the values between group 1 and *perse* group 3 (Fig. 2C-E).

Ameliorative effect of VIS on inflammatory cytokines

The concentration of pro-inflammatory cytokines (TNF-α, IL-1β, IFN-γ, IL-6, TGF-β and NF-Kb), in the liver tissue of DSS-treated group 2 was significantly (P<0.001) elevated compared to the untreated group 1. Conversely, treatment with VIS in groups 4, 5 and standard group 6 showed significant (P<0.05, P<0.001 and P<0.001, respectively) reductions compared to group 2, with group 5 exhibiting a more significant (P<0.05) reduction than group 4. Pro-inflammatory cytokine levels in group 5 were comparable to the standard group 6 with no significant difference. Group 3 revealed similar cytokine levels to the normal control group 1 indicating increased inflammatory markers in the DSS induced UC-mediated hepatic damage (Fig. 3A-F).

Additionally, the mean values of anti-inflammatory cytokine IL-10 levels were significantly (P<0.001) reduced in group 2 compared to group 1 and group 3 on the 32nd day of the experimental period. In contrast groups 4, 5 and 6 exhibited significantly (P<0.001) higher values compared to group 2 with group 5 showing significantly (P<0.05) more improvement than group 4 in a dose-dependent manner. Whereas, groups 5 and 6 exhibited almost non-significant mean values. No significant disparity was noticed between groups 1 and 3 (Fig. 4G).

Ameliorative effect of VIS on Gross pathology

Mice in group 1 and group 3 revealed grossly normal liver appearance. In contrast, group 2 mice showed severe congestion. Group 4 exhibited moderate congestion while groups 5 and 6 showed only mild congestion on the 32nd day of the experiment (Fig. 4).

Ameliorative effect of VIS on histopathology

To assess UC induced hepatic damage at cellular level, histopathological examination was done. Liver sections from group 1 and group 3 mice showed normal architecture including well defined hepatic cords, portal triad, sinusoidal space and central vein (CV) (Fig. 5A, E). In group 2 severe sinusoidal dilation and congestion of CV were evident. Hepatocytes showed necrosis, vacuolar degeneration with multifocal infiltration of MNCs (Black arrow) with pyknotic and karyorrhexis of nuclei along with swollen hepatocytes (Fig. 5C, D). Additionally, there was fibroblast proliferation in the hepatic parenchyma and distorted hepatic cords (Fig. 5B, red arrow). The liver section of VIS-treated groups 4 and 5 revealed moderate to mild degenerative changes indicating restoration of damaged hepatocytes (Fig. 5F, G). The liver sections of group 6 mice showed normal hepatic parenchyma with

only mild CV congestion (Fig. 5H).

Ameliorative effect of VIS on immunohistochemical expression

NF-κB is a key regulator of genes involved in the expression of pro-inflammatory mediators. Liver sections from groups 1 and 3 showed no immune reactivity for NF-κB protein (Fig. 6A, C). In contrast, sections from group 2 revealed strong positive immunoreactivity for NF-κB on the 32nd day of the experiment (Fig. 6B). Sections from group 4 showed moderate positive immunostaining for NF-κB protein indicated by moderate brown coloration in the sections while groups 5 and 6 exhibited mild positive immunostaining for NF-κB, indicating further reduction in the inflammation in these groups (Fig. 6D-F).

Nrf2 protein expression was assessed to evaluate the antioxidant enzyme activity in the liver. The expression of Nrf2 in group 2 showed a negative immunostaining (Fig. 6B) compared to group 1 and group 3 which showed intense positive immunostaining indicating strong antioxidant profile (Fig. 7A, C). Groups 4 and 5 showed mild to moderate positive immunoreactivity for Nrf2 protein, indicating a dose dependent enhancement in antioxidant status. Treatment group 6 exhibited a similar expression pattern as seen in high-dose VIS-treated group 5 (Fig. 7D-F).

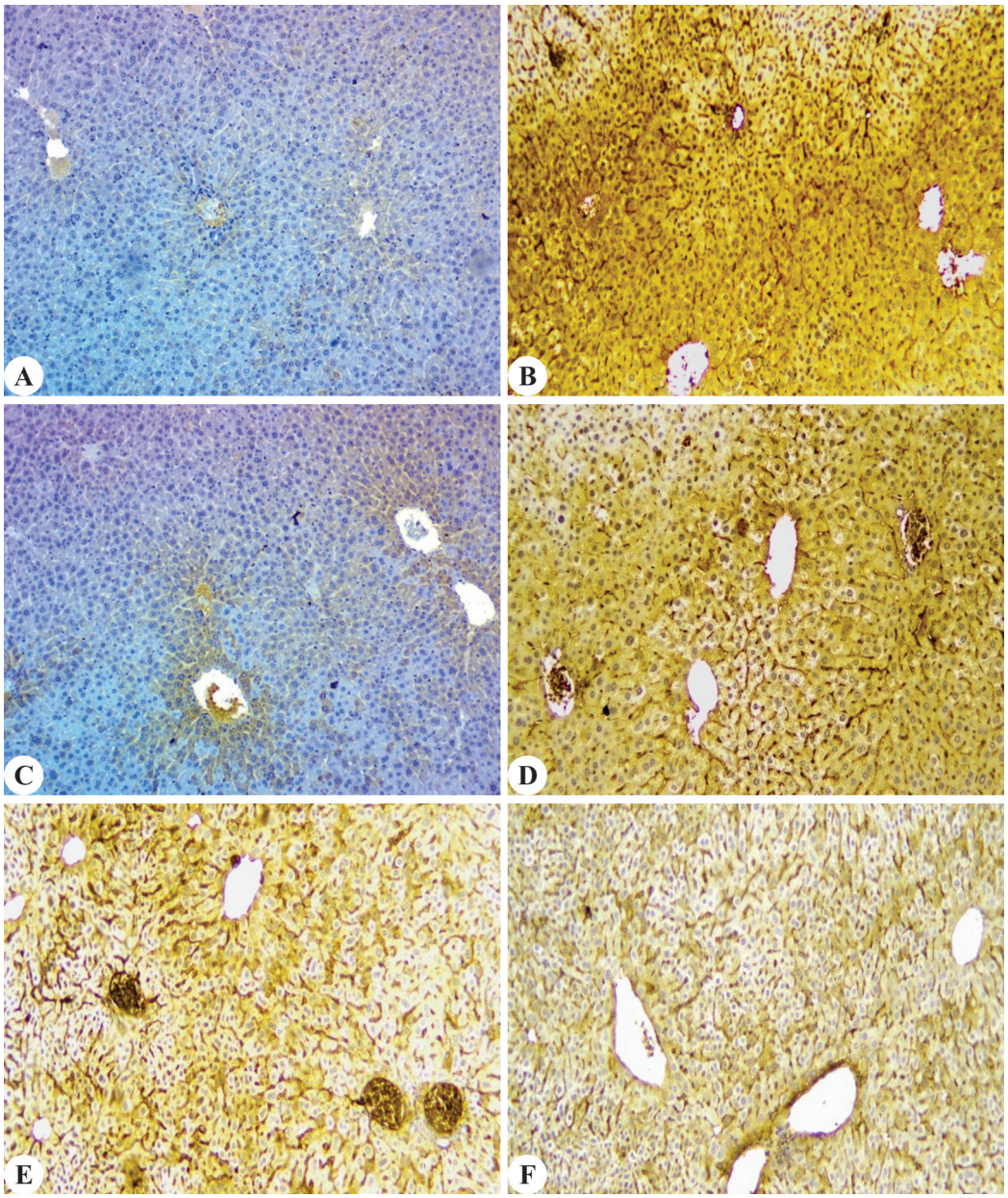

Fig. 6. Effect of VIS on immunochemistry of NF-κB marker in liver tissue. **A.** Control mice (G-1). **B.** DSS treated group (G-2). **C.** Ameliorative group (G-3). **D.** Low dose (G-4). **E.** High dose (G-5). **F.** Standard drug (G-6) (IHC X10).

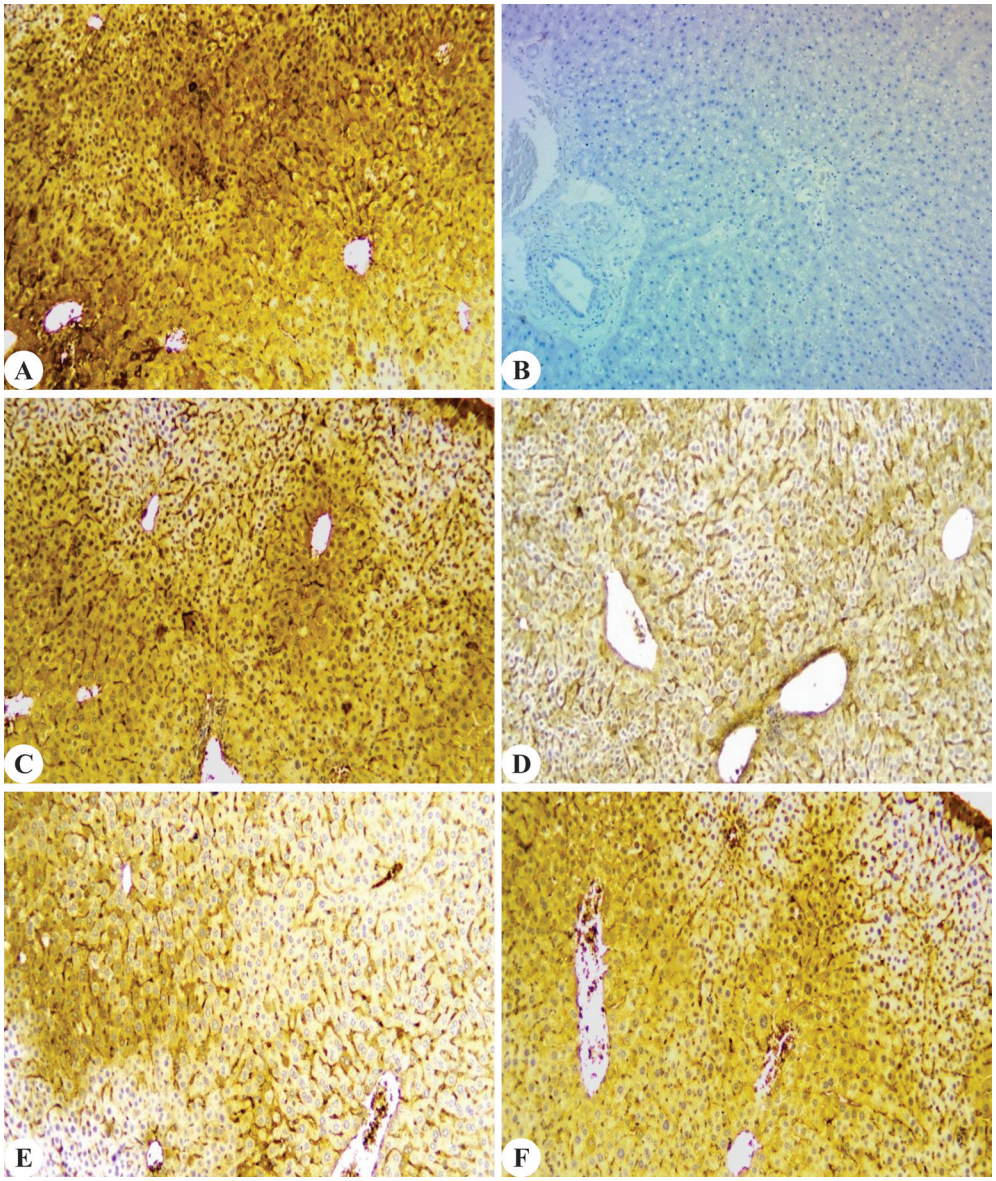

Fig. 7. Effect of VIS on immunochemistry of Nrf2 marker in liver tissue. **A.** Control mice (G-1). **B.** DSS treated group (G-2). **C.** Ameliorative group (G-3). **D.** Low dose (G-4). **E.** High dose (G-5). **F.** Standard drug (G-6) (IHC X10).

DISCUSSION

This study, investigates hepatotoxicity as anextra-intestinal manifestation of ulcerative colitis (UC) in mice and potential amelioration effect of VIS. The induction of UC in the mice was confirmed by a marked increase in the Disease Activity Index (DAI), along with significant cellular damage and inflammatory infiltration in the colon. Chronic UC is known to cause bacterial translocation to the liver, resulting in hepatic damage, which can be mitigated by use of natural plant products[16]. Previous studies have confirmed significant increased endotoxins levels and subsequently increased hepatic inflammation in mice with DSS-induced UC. The influx of by products of gut microbiota like LPS into the liver occurs due to compromised gut wall integrity and permeability. This activates liver derived inflammatory factors mainly toll-like receptor-4, which in turn activates NF-κB and triggers the secretion of pro-inflammatory cytokines ultimately leading to hepatic damage[16,17]. While several studies have examined the protective effects of natural agents against hepatotoxicity, we believe this study is first to investigate VIS potential to mitigate UC induced liver damage, focusing on oxidative stress, inflammation, apoptosis and DNA damage.

ALT and AST enzymes present in the hepatocytes are sensitive markers for assessing liver function and diagnosing hepatotoxicity[18]. Typically, absent in the serum, elevated levels of these enzymes in serum are sensitive indicators of liver and intestinal damage. In the present study, there was a significant increase in the levels

of serum ALT and AST in group 2, which might be due to increased bacterial translocation through altered TJ proteins, leading to hepato-biliary damage and impaired hepatic function[16,17].

Estimation of serum TP is a routine test used to assess the toxicological nature of the various xenobiotics. A significant reduction in TP and albumin levels in group 2 may be due to DSS-induced hepatocellular damage, resulting in changes in protein and free amino acid metabolism and their synthesis in the liver compared to control group 1. The reports of the present study were corroborated by previous reports[18]. Trivedi and Jena, 2013 studied DSS-induced liver injury and reported that destruction of the gut barrier results in increased bacterial translocation, which is associated with a significant increase in LPS into the portal vein blood flow and systemic circulation[17,19]. This, in turn, is reported to promote the release of pro-inflammatory cytokines, cellular damage and loss of functional integrity of hepatocytes, resulting in liver injury accompanied by significantly elevated levels of these enzymes. Conversely, VIS exhibited dose-dependent significant decline in these liver enzymes in groups 4 and 5 and in the standard group 6, with a significant improvement in TP and albumin levels compared to group 2. There was significant improvement observed in group 5 compared to group 4. However, group 5 and group 6 showed no significant difference. This might be due to the protective effect of VIS on damaged liver cells induced by toxic substances through its antioxidant properties, which help to neutralize free radicals and reduce oxidative stress in liver cells. This reduction leads to decreased hepato-cellular injury and hence lower levels of ALT and AST, and higher levels of TP and albumin suggesting hepatoprotective effects of VIS[20].

Systemic inflammation associated with UC is also responsible for causing oxidative stress in the liver due to the extra-intestinal manifestation of systemic circulatory mediators[21]. To examine the role of oxidative stress in hepatocellular damage, MDA and nitrite levels were measured showing a significant increase in oxidative stress in DSS induced group 2, potentially contributing to the hepatic damage and linked with severe tissue damage in previous studies. Additionally, oxidative stress has been implicated in both local and systemic DNA damage associated with UC. Excessive ROS levels can be nutritionally modulated by the supply of antioxidant substances that fuel the cellular antioxidant machinery, which comprises SOD, CAT and glutathione enzymes[22]. Antioxidant profile modulation is regulated by nuclear erythroid related factor-2 (Nrf2) protein, which helps to maintain the cellular homeostasis. Treatment with VIS significantly reduced lipid peroxidation and nitrosative stress by restoring antioxidant enzyme levels through Nrf2 signalling pathway[23].

The TLR4/NF-κB signaling pathway plays a critical role in the immune response, particularly in the development of immune-related liver injury[16,17]. Toll-like receptor 4 (TLR4), a key member of the toll-like receptor (TLR) family, play an important role in initiating inflammatory processes. LPS and cytokines can induce inflammation by activating TLR4 on macrophages, thereby exacerbating hepatic disorders. Additionally, TLR4 activation triggers the NF-κB signaling pathway, leading to the phosphorylation of p65 and IκBα in liver tissues, which in turn stimulates an inflammatory response that contributes to liver injury[18,19]. These insight combined with and research, reveals that VIS significantly reduces the expression of inflammatory proteins, and inhibits the phosphorylation of NF-κB pathway. This suggests that VIS may mitigate colitis-induced liver injury by modulating the TLR4/NF-κB/Nrf2 signaling pathway.

A significant elevation in pro-inflammatory cytokines levels in the liver of animals with UC indicates the role of inflammation in UC-associated liver damage contributing to oxidative stress and apoptosis in the liver. Additionally, NF-κB expression is significantly increased in DSS-treated group which is involved in expression of various inflammatory cytokines. IL-10, an anti-inflammatory cytokine suppresses T lymphocytes and mononuclear cells, reducing pro-inflammatory cytokines by inhibiting suppressor of cytokine signaling-3 and IκB kinases, thus blocking the NF-κB signaling pathway. A significant reduction in IL-10 levels was observed due to severe oxidative stress and release of pro-inflammatory cytokines from damaged tissue. Our results were in agreement with the previous studies showing significant down-regulation of IL-10 cytokine expression in experimental colitis of group 2 mice[17,18].

Administration of both low and high doses of VIS in groups 4 and 5 showed a significant increase in IL-10 levels and significant reduction in pro-inflammatory cytokines compared to group 2, indicating the anti-inflammatory effect of VIS, which is mediate by reducing NF-κB activation. Our findings further support this, as decreased NF-κB levels were observed. These results suggest that VIS effectively suppress inflammation in DSS induced liver injury[24].

Histopathological evaluation was done in order to assess DSS-induced hepatic damage at cellular level. Previous studies have shown that chronic inflammation of colon results into abnormal hepatic damage and degenerative changes[16,18]. Increased oxidative stress and inflammation are responsible for hepatic damage in DSS-induced UC. The liver sections of VIS treated groups 4 and 5 revealed moderate to mild degenerative changes

indicating the restoration of damaged hepatocytes in a dose-dependent manner. Further, the liver sections of group 6 mice showed normal hepatic parenchyma with only mild CV congestion.

NF-κB is a crucial gene, in the transcription of various pro-inflammatory mediators, involved in the development of hepatocellular damage. The DSS treated liver showed increased intensity of immunoexpression of NF-κB and decreased expression of Nrf2 likely due to the activation of inflammatory signaling pathway[15]. However, group 4 and group 5 showed moderate to mild expression suggesting increased activation of antioxidant pathway by VIS and group 6 also showed mild expression due to anti-inflammatory effect of dexamethasone.

In summary, the study concludes that DSS induced hepatic damage is marked by alteration in intestinal barrier integrity and a pronounced increase in the expression of pro-inflammatory cytokines, facilitated through the upregulation of the NF-κB. Additionally, there was a notable alteration in oxidative stress parameters attributed to downstream regulation of the Nrf-2 pathway. Administration of VIS suppresses oxidative stress and inflammation through the downstream regulation of NF-κB and upstream regulation of the Nrf-2 pathway in a dose-dependent manner. Additionally, the higher VIS dose produced results comparable to standard drug-dexamethasone suggesting that VIS may offer therapeutic potential for UC related hepatic injury. Further, molecular studies are needed to further elucidate the mechanism of extra-intestinal manifestations of UC and the protective action of various phytochemicals.

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