Evaluation of Therapeutic Effects of Vardenafil Targeting Nrf2 and NLRP3 Inflammasomes in a Rat Model of Ulcerative Colitis

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Abstract

Aims: Ulcerative colitis (UC) is a chronic relapsing inflammatory bowel disorder that known to increase the risk of colon cancer. Erectile dysfunction(ED) is common in patients with UC. Vardenafil, which is a drug commonly prescribed for treatment of ED, has been shown to have antioxidant effects; nevertheless, its significance in UC has not been determined. Thus, this current study was designed to investigate the potential therapeutic effects of vardenafil against acetic acid-induced UC and to identify possible underlying mechanisms.
Main methods: Induction of UC was accomplished using 3% acetic acid (AA) instilled rectally. In total, 45 male Wistar rats were classified into 5 groups: control group, UC group, sulfasalazine (100 mg\kg\day) group vardenafil (10 mg\kg\day) group, and combined sulfasalazine and vardenafil group.
Key findings: Vardenafil, alone and in combination with sulfasalazine, significantly ameliorated the severity of UC as indicated by reduced colon weight/body weight ratio, colon weight /colon length ratio, and macroscopic and microscopic scores of UC. Vardenafil significantly reduced oxidative stress as evidenced by decreased malondialdehyde abundance and increased colonic superoxide dismutase, nuclear factor erythroid-2-related factor-2, and heme oxygenase_1 concentrations. Moreover, treatment with vardenafil significantly suppressed NLRP3 inflammasome signaling pathway as indicated by decreased colonic concentrations of NOD-like receptor 3, caspase-1, and interleukin 1 beta.
Significance: Our findings prove that vardenafil has anti-inflammatory and anti-oxidant effects against AA-induced UC through activating Nrf2 signaling and suppressing NLRP3 inflammasome activation. Thus, vardenafil may represent a promising therapeutic candidate for treatment of UC especially for UC patients suffering from ED.
Keywords: ulcerative colitis, erectile dysfunction, vardenafil, Nrf2/Ho-1, NLRP3 inflammasome

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Introduction:

Ulcerative colitis (UC) is a chronic, relapsing inflammatory bowel disorder that has been linked to an increased risk of colorectal cancer. It is believed that several genetic and environmental factors have a role in its pathogenesis **(El-Baz *et al.*, 2020)**. Genes and proteins that regulate

oxidative and electrophilic stress, redox reactions, and inflammation are among the genetic variables that contribute to UC **(Piotrowska *et al.*, 2021).**

Nuclear factor erythroid 2-related factor -2 (Nrf2) has been identified as a key transcription factor which plays a pivotal role in cellular defenses against oxidative insults by induction of cytoprotective enzymes. Heme oxygenase-1 is one of the enzymes upregulated by Nrf2 that has obvious anti-inflammatory and anti-oxidative properties **(Kim *et al.*, 2010)**. Previous studies have revealed that activating Nrf2 signaling protects against experimentally induced UC **(Khodir *et al.*, 2019; Serrya *et al.*, 2021).**

The inflammasome is a well-defined protein complex found in the cytoplasm. It is a part of the innate immune response to cellular stress or infection **(Serrya *et al.*, 2021)**. The most well-studied inflammasome is the NOD-like receptor 3 (NLRP3) inflammasome, that has been linked to various inflammatory and autoimmune diseases **(He *et al.*, 2016)**. Uncontrolled activation of the NLRP3 inflammasome is a major contributor to IBD pathogenesis; hence, inflammasome inhibitors may have a bright future in the development of innovative approaches to IBD treatment **(Chen *et al.*, 2021).**

Phosphodiesterase type 5 inhibitors (PDEI5) represent agents that are commonly prescribed for treatment of erectile dysfunction (ED) and pulmonary hypertension **(El-Agamy *et al.*, 2018)**. PDEI5 have been reported to counteract many inflammatory responses in several animal models of inflammation as colitis and hepatitis **(Labib *et al.*, 2017; Manna *et al.*, 2017) and (Ahmed *et al.*, 2017; El-Agamy *et al.*, 2018)**.

In a cohort study, **Friedman *et al.* (2018)** found that those with IBD are more likely than men without IBD to be prescribed an ED medication. Another study conducted by **Shmidt *et al.* (2019)** demonstrated that the majority of men with recently diagnosed IBD suffer erectile dysfunction. **Sutton et al. (2020)** recently revealed that PDEI5 use in individuals with ED has been associated with a lowered risk of colorectal cancer compared to those who were not exposed to PDEI5.

Based on the findings presented above, we hypothesized that vardenafil, which is a commonly prescribed drug for ED, might represent an effective treatment option in models of UC. Thus, this study aims to assess the possible therapeutic effects of vardenafil administration alone or in combination with sulfasalazine in an experimental rat model of UC with morphological and molecular similarities to the human disease. If additive effects existed, patients may be given modest dosages of each treatment that are below the threshold for side effects while still providing maximal symptom relief. Combination therapy would be advantageous for UC patients suffering from ED, and, if the drug is shown to have good therapeutic results on its own, it could be an attractive choice for treating both illnesses while avoiding the negative effects of combination therapy.

Materials and methods

1. Experimental animals

In total, 45 male Wistar rats (200–230 gm in weight) were purchased from Zagazig University's Faculty of Veterinary Medicine (Egypt). Rats were placed in the Zagazig Faculty of Medicine animal house under full hygienic and standard environmental conditions for 1 week for acclimatization before commencing experiment. Free access to well-balanced water and diet was allowed. The experimental design and animal handling performed in this study were in compliance with the National Institutes of Health and the ARRIVE guidelines and were approved by Zagazig University's Institutional Animal Care and Use Committee (ZU-IACUC/3/F/144/2019).

2. Drugs and chemicals

Vardenafil and sulfasalazine were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Acetic acid (AA) used to induce UC was bought from El-Gomhouria chemical company (Zagazig, Egypt). All other chemicals utilized were of analytical grade obtained from Sigma-Aldrich.

3 .Experimental design

Rats were randomly assigned to one of five experimental groups (each with nine rats): normal control group (control), received distilled water orally (once daily for 7 successive days) 5 minutes after normal saline (0.9%) colonic instillation; UC group, received distilled water orally (once daily for 7 successive days) 5 minutes after instilling AA into the colon; sulfasalazine (sulfa) group, received sulfasalazine (100 mg\kg\day) **(Fouad *et al.*, 2021)** orally for 7 consecutive days 5 minutes following AA colonic instillation; vardenafil (vard) group, received vardenafil (10 mg/kg/day) **(Aziret *et al.*, 2014)** orally for 7 consecutive days 5 minutes following AA colonic instillation; and sulfasalazine + vardenafil (sulfa + vard)-treated group, received sulfasalazine (100 mg\kg\day) + vardenafil (10 mg\kg\day) orally for 7 consecutive days **(Manna *et al.*, 2017)** 5 minutes after colonic AA instillation .

4 .Induction of UC

To induce UC, rats were deprived overnight while having unlimited access to water. Under light anesthesia using thiopental sodium (20 mg\kg, I.P.), 5 ml/kg of AA (3%v/v) was supplied transrectally to rats using a soft pediatric polyethylene catheter with an exterior diameter of 2 mm that was placed rectally into the colon for 8 cm. To avoid acetic acid leakage from the colon, the rats' heads were sustained in a downward position for two minutes. The same procedure was performed on control animals, but an equivalent volume of normal saline was used instead of acetic acid **(Mascolo *et al.*, 1995; Jedidi *et al.*, 2021)**.

5 .Scarification and preparation of colonic tissue samples:

Rats were fasted the night before scarification. 24 hours after last dosing, animal weights were measured just before scarification using digital weighing scales; animals were thereafter

sacrificed by cervical dislocation under profound anesthesia with sodium thiopental (40 mg\kg, I.P) **(Khodir *et al.*, 2019).** Longitudinal abdominal incision was performed, and the whole colon was rapidly dissected to the proximal terminus, washed with saline, and cleaned of fat and mesentery. Colon weight and length of each rat were measured after animal scarification to estimate colon weight /body weight (CW/BW) ratio as an indicator of disease activity index **(Khodir *et al.*, 2019)** and colon weight /colon length (CW/CL) ratio as a simple and dependable indicator of intestinal inflammation and damage **(Fouad *et al.*, 2021)**.

The colon was scored macroscopically; then, the proximal 5 cm was preserved in formalin 10% for histopathological assessment, while the distal 5 cm was snap-frozen in liquid nitrogen and referred to biochemical analysis.

6 ·Macroscopic examination:

Evaluation of the morphologic injury was done according to the scoring system described by **Millar *et al.* (1996)** as follow; 0,No macroscopic change; 1, Mucosal erythema alone; 2, Mild edema of mucosa, slight bleeding or tiny erosions; 3, moderate edema, mild bleeding ulcers; 4, Severe ulceration, extensive edema and tissue necrosis. To eliminate bias, inspection and scoring were performed using a magnifying glass by two distinct observers who were blinded to each other **(Saber *et al.*, 2021).**

7 ·Histopathological examination:

Colonic specimens were fixed in formalin10% for 24 hours, embedded in paraffin, sliced into four-millimeter sections, and stained with hematoxylin and eosin. The grade of colon inflammation was assessed according to the scoring system reported by **Fabia *et al.* (1992)** and **Javed *et al.* (2016).**

8 ·Preparation of colonic homogenate:

Colon tissues were rinsed in phosphate buffered saline solution, (pH 7.5), containing 0.16 mg/ml heparin for removal of any RBCs or clots. The tissues were homogenized in 5–10 ml cold buffer (7.5 pH, 50 mM potassium phosphate) for gram tissue. Then, the homogenates were centrifuged, for 15 minutes at 4°C, at 100,000 × *g*. The supernatant was separated for subsequent assays and stored on ice.

9 ·Estimation of superoxide dismutase (SOD) and MDA levels in colon homogenates

SOD levels were estimated according to procedure reported by **Nishikimi *et al.* (1972)** using ELISA kits (MyBioSource, Inc. San Diego, CA, USA). MDA concentration was evaluated in colon homogenates, as an indication of lipid peroxidation, as described by **Ohkawa *et al.* (1979)** using ELISA kits purchased from Lifespan Biosciences Inc., Seattle, WA, USA.

10 ·Estimation of antioxidant Nrf2 pathway biomarkers in colon homogenates

Nrf-2 and Heme oxygenase-1 (HO-1) concentrations were assessed using ELISA assay kits (Cusabio Technology LLC., USA and MyBioSource, Inc., respectively) according to the manufacturers' instructions.

11 ·Estimation of NLRP3 inflammasome pathway concentrations in colon homogenates:

NLRP3, caspase_1 and IL-1β colonic concentrations were estimated using ELISA assay kits (My BioSource, Inc.) in accordance with the instructions of the manufacturer.

12 ·Statistical analysis

The obtained results were tabulated as mean ± SE. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test **(Elshazly *et al.*, 2020)**. Statistical analysis was done using GraphPad Prism v.5.02 (GraphPad Software Inc.; San Diego;CA, USA).

Results

Effect of sulfasalazine or/and vardenafil on CW/BW and CW/CL ratios in AA-induced UC

As per the findings of this study, UC rats exhibited a significant increase in CW/BW and CW/CL ratios (9.833 ± 0.456 mg/gm, and 303.7 ± 6.789 mg/cm, respectively) compared to control group animals (4.478 ± 0.157 mg/gm, and 96.69 ± 3.66 mg/cm, respectively). Treatment with sulfasalazine, vardenafil, or in combination has significantly decreased CW/BW and CW/CL ratios to (6.789 ± 0.301, 6.933 ± 0.365, and 5.144 ± 0.25 mg/gm, respectively) and (164.6 ± 8.086, 158.9 ± 7.4, and 115.9 ± 6.017 mg/cm respectively) compared to the UC group. No significant difference was observed between sulfasalazine- and vardenafil-treated groups, but more statistically significant improvement was evident with their use in combination (Table I).

Table I: Effects of sulfasalazine, vardenafil, and their combination on colon weight/body weight (CW/BW) ratio, colon weight/colon length (CW/CL) ratio, morphological injury score, and microscopic score

Groups	CW/BW ratio(mg/gm)	CW/CL ratio(mg/cm)	Morphological injury score	Microscopic score
Control group	4.478 ± 0.157	96.69 ± 3.66	0.22±0.147	0.56±0.176
Ulcerative colitis	9.833 ± 0.456 *	303.7 ± 6.789 *	3.89±0.111*	9.33±0.5*
Sulfasalazine	6.789 ± 0.301 *#	164.6 ± 8.086 *#	2.11±0.26*#	3.89±0.42*#

Groups	CW/BW ratio(mg/gm)	CW/CL ratio(mg/cm)	Morphological injury score	Microscopic score
Vardenafil	6.933 ± 0.365 *#	158.9 ± 7.4 *#	2.44±0.176*#	4.22±0.36*#
Sulfasalazine +Vardenafil	5.144 ± 0.250 #$@	115.9 ± 6.017 #$@	0.67±0.166#$@	1.89±0.35#$@

Each group's values are presented as the mean ± SE of nine rats.
*Significant difference versus normal control.
#Significant difference versus UC group.
$ Significant difference versus sulfa group.
@Significant difference versus vard group .

Effect of sulfasalazine or/and vardenafil on SOD and MDA levels in colonic homogenates

UC significantly decreased colonic SOD concentration compared to the control group (10.20 ± 0.657 U/gm vs. 43.78 ± 1.864 U/gm). Treatment with sulfasalazine, vardenafil, or in combination has significantly increased colonic SOD concentrations to 25.38 ± 1.36, 23.58 ± 1.39 and 39.18 ± 1.69 U/gm, respectively, compared with the UC group. There was no significant difference between sulfasalazine- and vardenafil-treated groups, but noticeably, greater statistically significant improvement was evident with their use in combination (Fig. 1, A).

UC significantly elevated the levels of colonic MDA, lipid peroxidation marker, as compared to the control group (35.57 ± 1.416 ng/gm and 4.044 ± 0.347 ng/gm, respectively). Treatment with sulfasalazine, vardenafil, or their combination significantly decreased colon MDA concentration to 20.06 ± 1.177, 21.21 ± 1.087, and 5.911 ± 0.525 ng/gm, respectively, compared with UC group animals. There was no significant difference between sulfasalazine- and vardenafil-treated groups, but, noticeably, greater statistically significant improvement was evident with their combination (Fig. 1, B).

Effects of sulfasalazine or/and vardenafil on Nrf2 antioxidant pathway levels in colonic homogenate

Concentrations of Nrf2 and HO-1 were determined to be significantly decreased in colons of UC rats compared to that in the control group (14.76 ± 0.761 pg/gm and 6.344 ± 0.768 ng/gm, respectively, vs. 48.53 ± 1.625 pg/gm and 38.57 ± 1.866 ng/gm). On the other hand, treatment with sulfasalazine, vardenafil, or their combination not only prevented depletion but also significantly increased Nrf2 and HO-1 concentrations compared to that in the UC group (30.60 ± 01.27, 27.44 ± 1.167, and 44.73 ± 1.74 pg/gm, respectively, and 22.28 ± 1.358, 19.21 ± 0.922, and 34.37 ± 0.295 ng/gm, respectively). Remarkably, sulfasalazine + vardenafil-treated rats exhibited the most significant increase in terms of Nrf2 and HO-1 levels compared with UC group animals (Figs. 1, C and D).

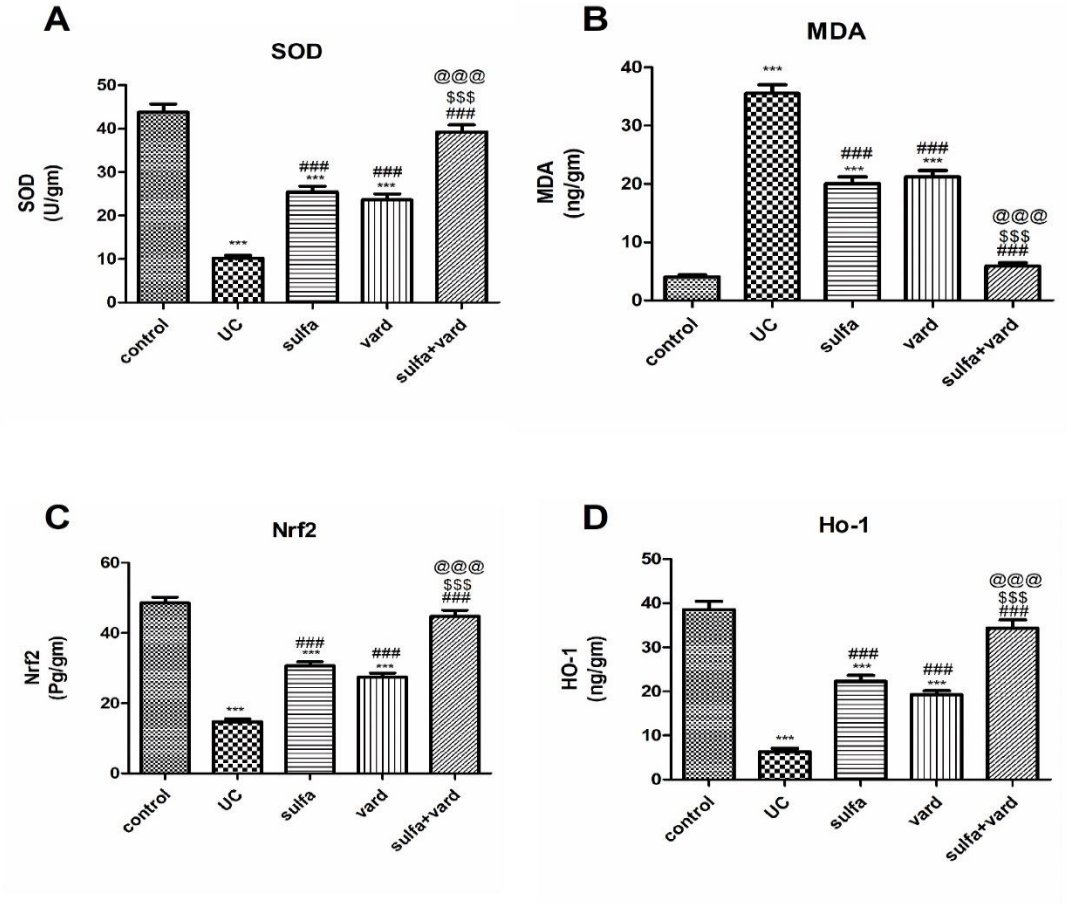

Fig. 1. Effect of sulfasalazine (100mg\kg), vardenafil (10mg\kg) and their combination on colonic concentrations of (A) Superoxide dismutase (SOD),(B) Malondialdehyde (MDA) (C) Nuclear factor erythroid -2-related factor- 2 (Nrf-2) and(D) heme oxygenase_1 (HO-1) . Data are presented as mean ± S.E, n = 9. ***P < 0.001 vs. Control,### P < 0.001 vs. UC group, $$$ P< 0.001 vs. Sulfa treated group, @@@ P < 0.001 vs. vard treated group.

Effects of sulfasalazine or/and vardenafil on NLRP3 inflammasome concentrations in colonic homogenates:

UC rats have exhibited significantly enhanced colonic NLRP3, caspase-1, and IL-1β concentrations as compared to those in the control group (18.90 ± 0.776 ng/gm, 48.34 ± 1.57 pg/gm and 46.69 ± 1.49 pg/gm, respectively) vs. (3.178 ± 0.47 ng/gm, 12.06 ± 0.969 pg/gm, and 10.64 ± 0.717 pg/gm).

On the other hand, treatment with sulfasalazine, vardenafil, or their combination significantly decreased colonic NLRP3, caspase-1, and IL-1β concentrations as compared to the UC group. The values were 10.68 ± 0.655 ng/gm, 29.50 ± 1.77 pg/gm, and 29.34 ± 1.26, pg/gm, respectively; 9.044 ± 0.472 ng/gm, 24.51 ± 1.718 pg/gm, and 26.32 ± 2.11 pg/gm, respectively; and 4.589 ± 0.718 ng/gm, 13.83 ± 1.3 pg/gm, and 12.31 ± 1.17 pg/gm, respectively) .

No significant difference was noticed between sulfasalazine- and vardenafil-treated groups; the same goes between the sulfasalazine + vardenafil-treated cohort and control group rats (Fig. 2).

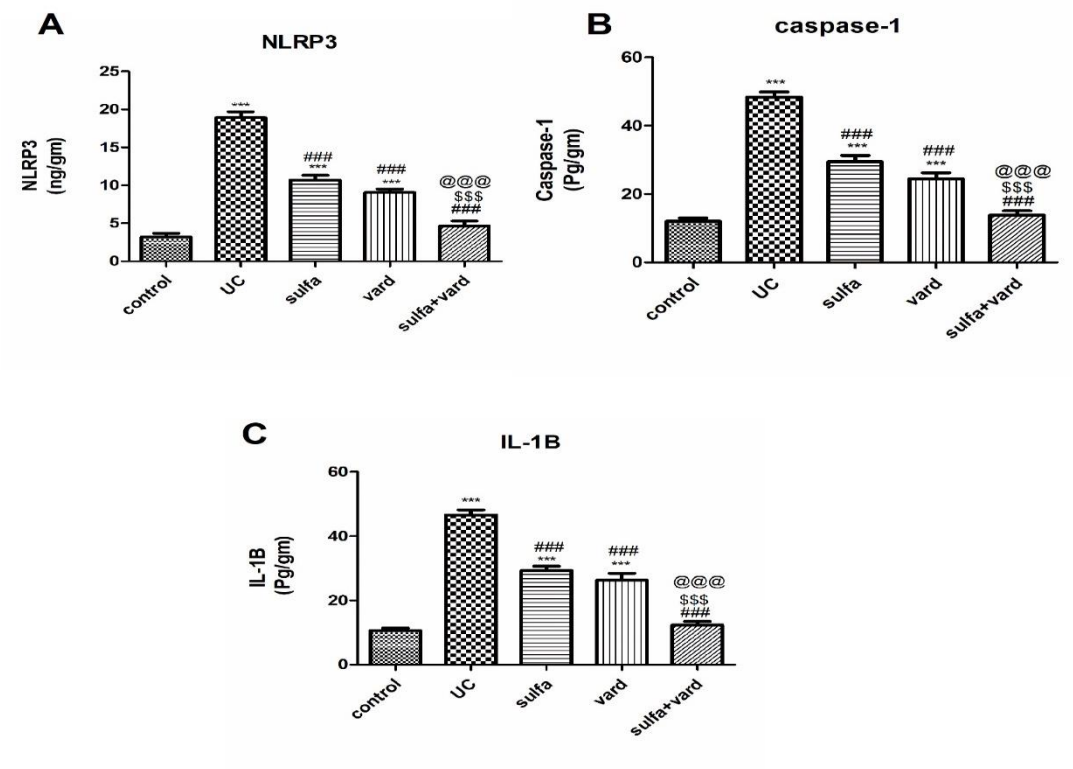

Fig. 2.Effect of sulfasalazine (100mg\kg), vardenafil (10mg\kg) and their combination on colonic concentrations of (A) The NOD-like receptor 3 (NLRP3), (B) caspase-1 and (C) Interleukin 1 beta (IL-1β). Data are presented as mean ± S.E, n = 9. ***P < 0.001 vs. Control,### P < 0.001 vs. UC group, $$$ P< 0.001 vs. Sulfa treated group, @@@ P < 0.001 vs. Vard treated group.

Effects of sulfasalazine or/and vardenafil on UC-induced pathological changes to the colon

1. **Effect on colitis morphological injury scoring**

In the control group, gross evaluation of the whole colon under a magnifying lens revealed normal smooth mucosa (Fig. 3 a). In the UC group, the colon showed extensive ulceration, edema, and tissue necrosis (Fig. 3 b), with a substantially higher macroscopic score of colonic damage compared to the normal control group. Meanwhile, vardenafil (10 mg\kg\day), sulfasalazine (100 mg\kg\day), and combination therapy-treated groups have markedly mitigated the morphological injury scores compared with the UC group (Fig.3c,d,e). No significant difference was identified between sulfasalazine- and vardenafil-treated groups, but noticeably, greater statistically significant improvement was evident with their combined use.

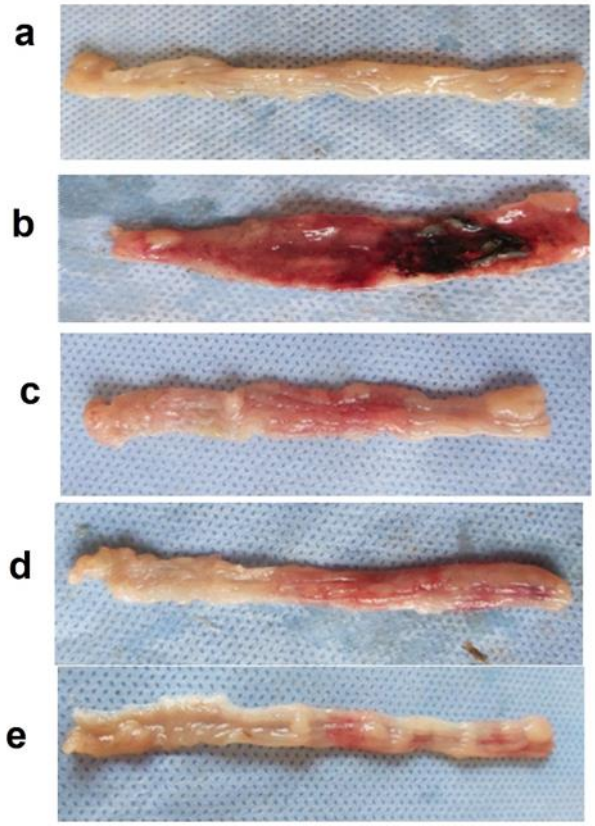

Fig. 3. macroscopic colonic appearance: control group showed normal smooth mucosa(a) ; UC group showed sever ulceration, edema and tissue necrosis(b) ; sulfa and vard groups showed mild mucosal edema, slight bleeding, and tiny erosions (c)and(d) respectively; sulfa + vard group showed mild mucosal redness and edema (e).

1. Effect on colitis microscopic scores:

Light microscopy of colon tissues from normal control group animals revealed normal mucosal architecture and muscle thickness. (Figs.4, A). The UC group had significantly disrupted mucosal architecture and severe inflammation infiltrating the mucosa and submucosa, as well as muscle layer thickening, crypt abscess formation, and goblet cell loss in the mucosal glands (Figs.4, B). On the other hand, treatment with either sulfasalazine or vardenafil or their combination markedly reduced the histopathological scores, compared with the UC group (Fig. 4 C, D,E). The colons in these groups had preserved mucosal architecture, mild to moderate inflammation within mucosa, and mild to moderate muscle thickening. No significant differences were noticed between sulfasalazine- and vardenafil-treated groups, whereas a greater statistically significant improvement was evident with their application in combination.

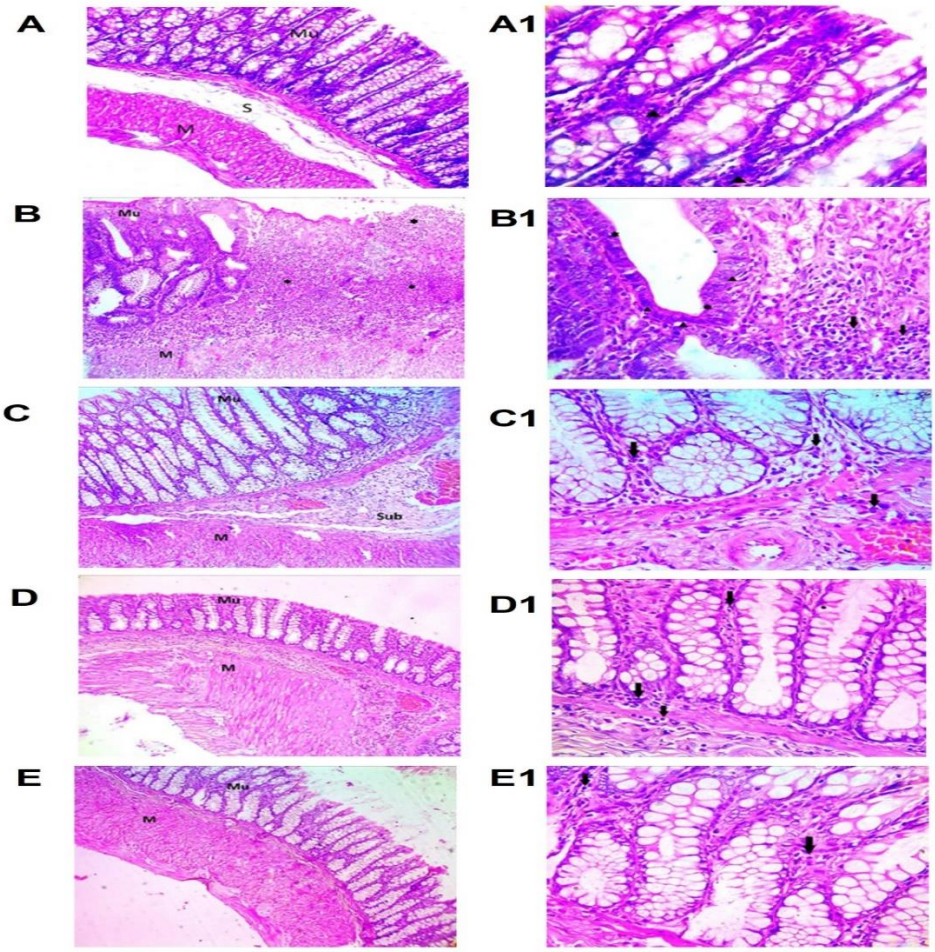

Fig. 4. Photomicrographs of slices of rat colons stained with H & E (100 and 400 magnifications, respectively).(A) Colon of control rats showing normal mucosal architecture, with normal muscle thickening.(A1) showing mild inflammation (arrowheads) infiltrating among mucosal glands. (B) Colon of UC rats showing marked loss of mucosal architecture, marked inflammation (asterisks) within mucosa and submucosa, marked muscle thickening.(B2) showing marked inflammation (arrows), reactive changes (nuclear enlargement, arrowheads) with loss of goblet cells (asterisks). (C) Colon of sulfa rats showing preserved mucosal architecture with glandular adenosis, moderate inflammation within mucosa and extended into submucosa, edema and congested blood vessels in submucosa and mild muscle thickening.(C1) showing moderate inflammation (arrows) within mucosa and extended into submucosa, no loss of goblet cells, no crypt abscess.(D) Colon of vard rats showing preserved mucosal architecture, moderate inflammation within mucosa and extended into submucosa, edema and congested blood vessels in submucosa, moderate muscle thickening. D1) showing moderate inflammation (arrows), no loss of goblet cells, no crypt abscess. (E) Colon of sulfa+ vard rats showing preserved mucosal architecture, mild inflammation, mild muscle thickening. (E1) showing mild inflammation (arrows), no loss of goblet cells, no crypt abscess. Mu: Mucosa, S: Submucosa, M: Muscle layer.

Discussion:

Ulcerative colitis is a chronic relapsing inflammatory bowel disorder which is known to not only reduce patients' quality of life but can also cause life-threating complications **(Khodir *et al.*, 2019)**.

This study was designed to test the possible therapeutic effects of vardenafil and its interaction with sulfasalazine against AA-induced UC in male rats. The results revealed that vardenafil obviously ameliorated the induced colitis in rats. UC was induced by instillation of AA rectally. This is one of the most commonly used UC models., as it is easily inducible, reproducible and closely matches human UC in terms of body weight loss, ulceration, and inflammatory influx **(Mohamed *et al.*, 2021; Serrya *et al.*, 2021).**

Wada-Hiraike *et al.* (2006) stated that estrogen can have a protective effect on colonic mucosa. According to **Nie et al. (2018),** estrogen is vital in maintaining mucosal barrier functions in the gastrointestinal system, including epithelial and physiological barrier functions. They stated that the lack of estrogen in postmenopausal women and in men is linked to higher level of reactive oxygen species (ROS) and higher risk of various diseases, including UC. Hence, in this current study, we selected male rats to avoid the protective effects of estrogen.

In this present study, rats subjected to rectal AA instillation developed colitis as evidenced by marked decrease in body weight, increased CW/BW ratio, and elevated CW/CL ratio compared to those of the control group .These results are supported by other previous studies (**Chorawala *et al.* 2021 ; Mohamed *et al.* 2021).**

Yilidrim *et al.* (2014) and Abdelmegid *et al.* (2019) have attributed the marked decrease in body weight to the inflammatory condition, which then leads to nutrient malabsorption, generalized catabolic state, catabolic cytokines (e.g., TNF), and alterations to the levels of metabolic hormones affecting satiety and thereby resulting in anorexia.

Kjellev *et al.* (2006) stated that the CW/CL ratio is a highly relevant and easily measured postmortem macroscopic marker of colitis that closely reflects both epithelial hyper proliferation and inflammatory cell influx during the course of UC disease progression. They also stated that with UC, colon weight increases due to tissue edema and cellular infiltration, whereas colon length decreases due to longitudinal muscle spasms.

Histopathological images of the colon revealed classic UC characteristics such as severe ulceration, edema, tissue necrosis, increased vascularity, significant inflammatory cellular infiltration, crypt abscess formation, and a significant decrease in goblet cell numbers. These findings are concordant with those of **El-Akabawy and El-Sherif (2019) and Jedidi *et al.* (2021),** who reported significant deleterious effects involving colon structure, macroscopically and microscopically, in an AA-induced rat model of UC.

Our work revealed that oral administration of vardenafil had the ability to alleviate AA-induced alterations in body weight, CW/BW ratio, CW/CL ratio, and macroscopic and microscopic scores. These outcomes suggested that vardenafil may have a promising therapeutic impact in the treatment of UC. These results are partially consistent with earlier researches that reported the potential therapeutic effects of sildenafil–another PDEI5–in experimentally-induced colitis models **(Karakoyun *et al.*, 2011; Manna *et al.*, 2017).** These authors ascribed their results to the potent anti-inflammatory and antioxidative effects of sildenafil along with its ability to inhibit adhesion molecule synthesis in colonic tissues. **Labib *et al.* (2017)** have also reported the

coloprotective effects of tadalafil against AA-induced UC, possibly through its anti-inflammatory, antioxidant, and apoptosis reduction effects.

ROS are partly responsible for tissue injury in many inflammatory diseases, including UC. When the rate of generation of toxic oxidants surpasses the capacity of endogenous antioxidative enzymes (e.g., superoxide dismutase and glutathione peroxidase), they can cause harm **(Grisham *et al.*, 1991; Iseri *et al.*, 2009).** Activated leukocytes and neutrophils are known to release reactive oxygen species in the inflamed mucosa, causing depletion of endogenous anti-oxidants and interaction with cellular lipids, resulting in lipid peroxidation **(Sokol *et al.*, 1991; Abd El Motteleb *et al.*, 2017).**

SOD catalyzes the decomposition of superoxide radicals to molecular O_2 and hydrogen peroxide, making it an important component of the cellular antioxidant defense mechanisms **(Fridovich, 1997; Serrya *et al.*, 2021).** Our results confirmed these findings as UC rats exhibited significant increases in MDA concentrations and decreased SOD concentrations compared with control group rats. These findings are consistent with those of **Bastaki *et al.* (2016)**, who found that intrarectal administration of 3% AA increased MDA levels and depleted colonic SOD concentrations. The authors stated that the underlying mechanisms involving AA-induced localized mucosal injury and desquamation are caused by acetic acid conversion to a protonated form, which, in turn, diffuses into the epithelial cells and later dissociates to form protons. This leads to intracellular acidification, induction of inflammation, and mobilization of granulocytes and macrophages to inflamed epithelial layers, which results in the overproduction of pro-inflammatory mediators and ROS.

This report showed that administration of vardenafil significantly decreased MDA concentrations and prevented depletion of SOD levels, suggesting that vardenafil has strong antioxidant activity, which may help to reduce oxidative damage and improve healing. These findings are consistent with those of **Aziret *et al.* (2014)**, who found that vardenafil administration decreased MDA levels in a dose-dependent manner. Similar results were obtained by **Labib *et al.* (2017)**, who found that tadalafil significantly lowered MDA abundance and elevated SOD levels in colonic tissues in a rat model of UC.

Nrf2 is a vital transcription factor regulating cellular responses against oxidative insults. Upon exposure to ROS, Nrf2 stimulates the expression of several cytoprotective enzymes such as SOD and HO-1 **(Kim *et al.*, 2010).** Nrf2 signaling loss or disruption, raises the risk of oxidative stress and tissue injury, whereas its activation reduces pathological inflammatory responses and tissue damage **(Serrya *et al.*, 2021).** This concurs with our results, where UC decreased colonic Nrf2 content and treatment with vardenafil increased such levels, suggesting that vardenafil's antioxidant benefits against UC may be mediated through Nrf2 upregulation and subsequently increased antioxidant enzyme expression.

HO-1 is cytoprotective agent which is activated by Nrf2. It catalyzes heme breakdown into bilirubin and carbon monoxide, which are known to have antioxidant and anti-inflammatory properties **(Puentes-Pardo et al., 2020).** Many studies have shown that increasing HO-1 expression may reduce tissue damage in UC models **(Yang *et al.*, 2017; Lin *et al.*, 2019).**

In this present study, vardenafil replenished colonic HO-1, which confirmed the cytoprotecive effect of HO-1 in experimental models of UC. These findings are in accordance with previous study showing that the hepatoprotective effect of vardenafil against cholestatic damage is mediated partly through its ability to upregulate hepatic expression of Nrf2 and HO-1 **(El-Agamy *et al.*, 2018).** Similarly, recent studies revealed the capability of other PDE-5 inhibitors to activate Nrf2 and HO-1 signaling pathways **(Abdel-Wahab *et al.*, 2020; Fang *et al.*, 2020; Song *et al.*, 2021).**

Activation of NLRP3 inflammasomes is known to play a critical role as a trigger of inflammatory responses in various inflammatory and autoimmune diseases including UC. NLRP3 activation is linked to overproduction of ROS and oxidative stress **(Chen *et al.*, 2021).** Activated NLRP3 inflammasomes catalyze conversion of procaspase-1 to active caspase-1, which then stimulates the maturation and release of IL-1β and IL-18 from precursors. Moreover, caspase 1 triggers pyroptosis, a pro-inflammatory form of programmed cell death. IL-1β is a key pro-inflammatory cytokine, directing inflammatory cells to sites of infection and inducing cytokine production, thereby leading to increased tissue injury **(Jin and Flavell, 2010; Perera *et al.*, 2018).** High expression of NLRP3 has been recorded in colons of other UC models **(Cao *et al.*, 2021; Serrya *et al.*, 2021).** Moreover, increased IL-1β levels have been reported in UC patients, and animal models and have been correlated to disease severity **(Tourkochristou *et al.*, 2019).** This is consistent with the results of this present study where UC increased colon NLRP3, caspase-1, and IL-1β levels. Treatment with vardenafil has markedly decreased NLRP3, caspase-1, and IL-1β abundance, suggesting that the anti-inflammatory effects of vardenafil against AA-induced UC may be mediated through NLRP3 inflammasome suppression. The results of this current study are in accord with those of **El-Agamy *et al.* (2018)**, who found that vardenafil protects against lithocholic acid-induced cholestatic liver damage through inhibition of the NLRP3-caspase 1-IL-1β axis. Other PDE5 inhibitors were found to decrease IL-1β levels in a rat model of UC **(Labib *et al.*, 2017)** and pancreatitis **(Fang *et al.*, 2020).**

A crosstalk and inverse relationship involving Nrf2 and NLRP3 pathway is present. However, the exact underlying molecular mechanisms are not entirely understood. Both pathways are linked to stress and inflammatory conditions. Nrf2 activation causes increased expression of proteins that then detoxify ROS, whereas ROS are known to activate NLRP3 inflammasomes. Therefore, activation of Nrf2 may cause NLRP3 inflammasome inhibition **(Hennig *et al.*, 2018; Chen *et al.*, 2019).**

It is worth noting that the ameliorating effect of vardenafil against AA-induced UC was insignificant compared to that of sulfasalazine, whereas a more statistically significant improvement was evident with their administration in combination.

Conclusion

In conclusion, this present study demonstrated the therapeutic potential of vardenafil against AA-induced UC through activating Nrf2 signaling and suppressing NLRP3 inflammasome

activation. Therefore, vardenafil may represent a promising therapeutic candidate for UC treatment, in particular for UC patients suffering from erectile dysfunction.

CRediT authorship contribution statement

Esraa Yehia: Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing read and approved the final manuscript.

Elsayed M Kamel: Conceptualization, Resources, supervision, read and approved the final manuscript.

Amal E Salem: Validation, Resources, Formal analysis, Writing – original draft, Writing – review & editing, read and approved the final manuscript.

Doaa M Abdallah: Statistical analysis, Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, read and approved the final manuscript.

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