AFRICAN JOURNAL OF PHARMACY AND ALTERNATIVE MEDICINE

https://doi.org/10.58460/ajpam.v3i01.129

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RESEARCH ARTICLE

MJM BIOLABS

Safety Profile and Antiulcerogenic Activities of *Cordia Vignei* Hutch & Dalziel (Boraginaceae) Leaf Extract Against Chronic DSS-Induced Colitis in C57BL/6 Mice

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Article History

Submitted: 26th December 2024

Accepted: 1st March 2025

Published Online: 26th March 2025

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ABSTRACT

Cordia vignei plant is used in folkloric medicine to treat numerous inflammatory disorders. Inflammatory bowel disease incidence rate is steadily increasing in developing countries and mostly characterized by recurrent inflammation along with major problems. The current study aimed to establish the acute oral safety of *Cordia vignei* leaf extract and evaluated its antiulcerogenic activities in DSS-induced colitis model in mice. The phytomedicinal efficacy of CVE (30, 100 and 300 mg/kg, *p.o*) on colitis was assessed by inducing 5% (w/v) dextran sodium sulphate [MW= 50 kDa] in mice. Clinical parameters including body and organ weights, macroscopic and histological examinations, hematological and biochemical analyses, and antioxidant activity were studied. We showed that *Cordia vignei* extract significantly improved body and colon weights, restored hematological indicators *(P<0.05)* and reduced disease activity index scores *(P<0.05)*. Additionally, *Cordia vignei* extract inhibited histoarchectural alterations in the colons and inhibited oxidative damage to the colon tissues *(P<0.05)*. Furthermore, *Cordia vignei* extract was found to be relatively safe. We therefore conclude that *Cordia vignei* extract exhibited ameliorative effect against dextran sodium sulphate-induced colitis in mice.

Keywords: *Cordia vignei* extract, dextran sodium sulphate, oxidative stress, ulcerative colitis, inflammation

INTRODUCTION

The gut, a vital organ in the human body, is where digestion, fermentation, and nutrient absorption occur. The intestinal barrier and mucosal layer of the gut protect the host from infections and toxic chemicals. Consequently, a healthy stomach serves as the cornerstone of human health (Wu et al., 2022). Currently, the intake of more processed foods in dietary patterns has rendered gut inflammation a significant hazard to human health (Brichacek et al., 2024). Around the globe, gastrointestinal food allergies and chronic idiopathic inflammation are prevalent, hence contributing to several systemic illnesses, including obesity and cancer (Jia et al., 2021).

Inflammatory bowel disease (IBD) is an umbrella term that encompasses disorders associated with persistent inflammation of the gastrointestinal structure, and the etiology of this condition is unknown (Zhang et al., 2024). The condition primarily comprises two subtypes: ulcerative colitis (UC) and Crohn's disease (CD). Ulcerative colitis predominantly destroys the outer layers of the intestines, while Crohn's disease generally affects the deeper tissues of the intestinal wall (Wu et al., 2022). There has been a significant increase in the prevalence of IBD in developing nations, and according to WHO figures, colorectal cancer accounted for 1.8 million new cases globally and led to 880,792 fatalities in 2018 (WHO, 2018; Sánchez-Guillén & Arroyo, 2020). Numerous investigations indicate that various elements, including environmental variations, dietary choices, pathogenic infections, and bacterial imbalances, might propel the advancement of gut inflammation (Zhang, 2022; Aden et al., 2019). Conventional IBD treatments, including antibiotics, biologics, and immunosuppressants, can entail a range of considerable adverse effects (Cai et al., 2021). The current trajectory of IBD etiological research has introduced the possibility of novel therapy methods, including naturally occurring chemicals (Kim et al., 2023). Consequently, it is progressively essential to investigate alternative candidate agents sourced from natural resources to manage IBD.

Many individuals assert that all plant-derived products are inherently safe and do not need safety evaluations (Alkahtani et al., 2022).

Therefore, it is essential to evaluate the safety of plant extracts for human ingestion before contemplating their possible medicinal applications. One efficient way to do this is to perform acute oral toxicity testing *in vivo* (Muñoz et al., 2021). As part of a cultural legacy, folkloric therapies are usually handed down from ancestors to subsequent generations. As a result, the general public frequently uses these treatments without worrying about their possible damage (Valéria Soares de Araújo Pinho et al., 2014). Concern over the possible toxicity of herbal medicine is developing as more people utilize therapeutic plants and their products. Therefore, determining the toxicity of therapeutic plants used in traditional medicine becomes essential to human safety (Rosidah et al., 2024). Acute and sub-acute toxicity assessments are routinely performed to evaluate natural substances or manufactured pharmaceuticals (Alelign et al., 2020; Osagie Eweka et al., 2021).

Cordia vignei (Hutch. and Dalziel; Fam. Boraginaceae) is a medicinal plant species commonly found in humid tropical regions such as West African countries, including Ghana. It is a tropical arboreal species that mostly grows to a height of 10 m and a diameter ranging from 30 cm to 40 cm (Burkill, 2004). Traditionally, the decoction of the leaves was used for wound healing and as a laxative. In addition, powdered leaves of the plant were applied to the body as a remedy for rheumatism (Burkill, 2004) and in conjunction with certain parts of other plants to treat prostate cancer (Agyare et al., 2017). We have earlier reported on its acute anti-inflammatory properties (Owusu et al., 2023) and acute colonic damage prevention (Owusu et al., 2020). Furthermore, the plant has been reported to contain some phytochemical constituents, including alkaloids, saponins, tannins, flavonoids, glycosides, and triterpenoids (Owusu et al., 2023). Despite reports of the plant's anti-inflammatory effects, there is currently no scientific evidence to confirm its therapeutic potential against chronic ulcerative colitis and determine its oral safety profile. Therefore, this current study sought to establish the oral safety of *Cordia vignei* leaf extract, and further evaluate its antiulcerogenic activity in a chronic dextran sodium sulphate-induced ulcerative colitis model in mice.

MATERIALS AND METHODS

Chemicals and reagents

Sulfasalazine tablets ((KAR LABS LTD, New Delhi, India)), Ethanol (Ernest Chemist, Accra, Ghana), Tragacanth (Pharm-Inter, Brussels, Belgium), DSS (MP-Biomedicals, California, USA)). All other chemicals and reagents were of commercial grade.

Plant leaves collection, authentication and extraction

Fresh leaves of *Cordia vignei* were harvested in September from the Diabaa Forest Reserve, situated at longitudes 3° W and 3° 30′ W, and latitudes 7° N and 7° 30′ N, in the Dormaa West District,

Brong Ahafo region, Ghana. Mr. Asare, a botanist, in the herbarium of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, authenticated the leaves. A voucher specimen (KNUST/HM1/2017/L003) was preserved in the herbarium. The plant material was air-dried at room temperature for seven days and 3.5 kg of the dried leaves was ground into a fine powder using a heavy-duty blender (2200W-1400RPM Heavy Duty Electric Blender, Zhengzhou Hongji Mining Machinery Co., Ltd. Henan, China). The fine powder was macerated with 5L of 70% (v/v) ethanol for 72h, then filtered and concentrated using a rotary evaporator (DW-RE-52AA Water Bath Rotary Evaporator, Chongqing Drawell Instrument Co., Ltd., Chongqing, China) at 50°C, and finally solidified in an oven (InfitekDON18E, DON-18 Oven, Infitek Inc., Spokane, WA, USA). A semi-solid dark green extract was obtained, final yield was 11.62% (w/w) and, later stored in a desiccator for 72h. The extract was reconstituted as an emulsion in 2% tragacanth for oral administration and designated as *Cordia vignei* extract (CVE) in this study.

Experimental design

Thirty-Six mixed sexes of C57BL/6 mice (9-10 weeks old; 20-25 g) and forty mixed sexes of Sprague-Dawley rats (9-10 weeks old; 150-200 g) were purchased from Center for Scientific Research into Plant Medicine, Akuapem Mampong, Ghana. The animals were randomly chosen, kept in stainless steel cages with softwood shavings bedding, and given a commercial pellet meal sourced from GAFCO Co., Ltd., Tema, Ghana. All animals had unimpeded access to water. Before the start of the experiment, the animals were given sufficient time to acclimatize to their new environment and were kept under normal settings of temperature (25±2°C), a 12-h light-dark cycle, and regulated humidity. All animals were handled as per the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (National Institutes of Health (NIH), US, Department of Health Services Publication no. 83-23, revised. 2011). Furthermore, protocols used for oral toxicity study were in accordance with Organization for Economic Co-operation and Development guidelines for acute and subacute oral toxicity testing for compounds (OECD, 2001). All animals who perished prior to the experiment's conclusion, together with those that were euthanized, were collected in a biohazard container and then kept at -20ºC for roughly 24 h before incineration.

Oral acute toxicity test of CVE

Forty rats (150-200g) were randomly selected into four groups (n=10) and acclimatized in test cages (47cm × 34cm × 37cm^3) for one week under the same conducive environmental condition. Animals were denied food overnight; however, water was made available ad libitum. Initial weights of the animals were taken before commencement of oral treatment. Animals received oral treatment of CVE at low dose (LD), moderate dose (MD) and high dose (HD) each by a single administration of 500, 1500 and 3000 mg/kg extract, accordingly. The control group orally received 10 mg/kg of normal saline. The rats were carefully observed at time intervals of 30 min, 1, 2, 3, 4, 5, 6, 8, 10, 12 hours and 24 h for any signs of toxicity related to behavior such as tremors, convulsion, sedation, aggression, and physiological symptoms including salivation, diarrhea, and change in skin colour, eyes, fur, stool, or mortality (Irwin, 1968; Obakiro et al., 2024). Lethal dose (LD_{50}) of CVE was estimated using the formula (Obakiro et al., 2024):

$$\text{Lethal Dose } (LD_{50}) = \sqrt{\frac{D_0 + D_{100}}{2}}$$

Where D_0 is the highest dose that caused no death; D_{100} is the minimum dose that caused death.

The surviving animals were monitored for an additional fourteen days, with daily observations conducted[19] by receiving repeated doses of CVE (500, 1000 and 1500 mg/kg) orally. The consumption of water and food were observed daily. At the conclusion of the experiment, all animals were subjected to overnight fasting, weighed and euthanized under pentobarbital (50 mg/kg, IP) anesthesia.

Change in body weight

The body weights of animals were measured immediately before the administration of the extract and every other day until the conclusion of the study (i.e. day 0, 2, 4, 6, 8, 10, 12, 14). The percentage change in body weight was determined using the formula:

$$\%\ \text{change in rats' body weight} = \left(\frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100\right)$$

Hematological and biochemical evaluation

Blood samples were obtained via cardiac puncture into Ethylenediaminetetraacetic acid (EDTA) tubes for hematological assessment. A complete blood count was analyzed using automated heam analyzer (BIOBASEBK-6310 Automated Blood Test Analyzer, Biobase Biozone Co., Ltd., Shandong, China). Serum was prepared by centrifuging (YSENMED-YSCF3024 High Speed Micro.

Table 1.
Treatments for mice in the various groups

Groups	Treatment
I	Received 10 ml/kg of normal saline (naive)
II	Received 5% DSS (7 days) + free water for 28 days (negative control)
III	Received 5% DSS (for 7 days) + Sulfasalazine (500 mg/kg, *p.o*, for 28 days)
IV	Treated with 5% DSS (for 7 days) + CVE (30 mg/kg, *p.o*, for 28 days)
V	Treated with 5% DSS (for 7 days) + CVE (100 mg/kg, *p.o*, for 28 days)
VI	Treated with 5% DSS (for 7 days) + CVE (300 mg/kg, *p.o*, for 28 days)

Centrifuge, Guangzhou Yueshen Medical Equipment Co., Ltd., Guangzhou, China) 4ml of blood samples at 3000 × g rpm for 10 min at 4 □. The sera were preserved at -80□ in Eppendorf tubes and biochemical evaluation was conducted using an automated chemistry analyzer (YSTE100G Auto Chemistry Analyzer, Guangzhou Yueshen Medical Equipment Co., Ltd., Guangzhou, China).

Relative organ weight

Vital organs, including the liver, stomach, and kidneys of the animals, were excised and weighed. The relative organ (i.e. liver, kidney and stomach) weight was estimated using the formula:

$$\text{Relative organ weight} = \left(\frac{\text{Organ weight (g)}}{\text{body weight of rat on sacrifise day (g)}} \times 100\right)$$

Histological examination

The liver, stomach and kidneys were harvested, cleaned and fixed in 10% (v/v) formalin saline. Tissues were processed, cut into 5µm thick sections and stained with hematoxylin and eosin stain (H&E) for histopathological investigation.

Effect of CVE on DSS-induced colitis

Six experimental groups were formed (Table 1); each group, including the control, consisted of six mice (n=6).

Induction of colitis

Colitis was induced by following the procedure previously described by Chassaing et al. (2014) with slight modification. Briefly, mice in randomly selected groups (six groups; n=6) were treated as summarized above (Table 1). Each cage water bottle was filled with 100 ml water containing 5% (w/v) freshly prepared DSS (MW=50 kDa). Chronic colitis was induced by supplementing their drinking water with 5% DSS for seven consecutive days starting from day 1, and daily treatment with either sulfasalazine (500 mg/kg) or CVE (30, 100 and 300 mg/kg) commenced from day 0 until the 28th day. All mice were humanely sacrificed on day 28 after anesthesia with 20 % pentobarbital sodium.

Determination of change in body weight

Changes in body weight (g) of mice (n=6) in all treatment groups were determined every four days until the end of the experiment. Changes in body weight were represented as percentages over the initial weights. The percentage change in body weight was determined.

Colon weight and length

On day 28, mice were euthanized by cervical dislocation under mild pentobarbital (20%, ip, n=6) anesthesia. Colons were excised and longitudinally incised to extract fecal waste, thereafter rinsed gently under running water. The opened colons were carefully straightened on non-absorbent surfaces, and their length and weight were blindly measured.

Macroscopic evaluation of the colons

The internal morphology of the dissected colons was assessed using a blind scoring system on a scale of 0 to 4: 0= no apparent change; 1= mucosal erythema only; 2= mild mucosal edema, minimal bleeding or erosion; 3= moderate edema, slight bleeding ulcers or erosions; 4= severe ulceration, edema, and tissue necrosis (Paine, 2014).

Microscopic assessment of the colons

Samples of the distal colons were promptly preserved in a 10% formaldehyde solution, embedded in paraffin, and sectioned to a thickness of 5µm. Subsequent to deparaffinization, the sections were stained with hematoxylin and eosin (H&E) stain. The severity of ulceration, leukocyte infiltration, edema, crypt loss, surface epithelial cell hyperplasia, goblet cell reduction, and epithelial degeneration were assessed blindly on a 0-4 scale (Zhang et al., 2024) using a light microscope (DM 750 with ICC 50 HD, Leica Microsystem, GmBH, Wetzlar, Germany).

Hematological analysis

Blood samples were obtained from the mice (n=6) across all treatment groups into EDTA tubes. A complete blood count examination was conducted using an automated hematology analyzer (BIOBASE-BK-6310 Automated Blood Test Analyzer, Biobase Biozone Co., Ltd., Shandong, China).

Assessment of antioxidant activity

Colon tissues harvested was homogenized and the homogenate was centrifuged (YSENMEDY SCF3024 High Speed Micro-Centrifuge, Guangzhou Yueshen Medical Equipment Co., Ltd., Guangzhou, China) at 10 °C at 4000 × g rpm for 4 minutes, and the supernatant was collected. The supernatants were then used for the assays of glutathione (GSH), myeloperoxidase (MPO), and malondialdehyde (MDA).

Statistical analysis

Results were presented as mean±SEM. Data with two independent variables were subjected to two-way ANOVA with Boferroni's *post hoc* test while those with single independent variable were analyzed using t-test. GraphPad Prism for Windows version 9.0 (GraphPad Software, San Diego, CA, USA) was used for all statistical analysis. $P<0.05$ was considered statistically significant.

RESULTS

Acute oral toxicity study

In the acute toxicity testing, all dosages of the extract (500, 1500 and 3000 mg/kg, *p.o*, n=6) showed no mortality or behavioral alterations in the rats over the study period. There were no noticeable signs of extract-induced toxicity in the 24-h as well as the 14-day period thus, the findings of the acute oral toxicity investigation suggested that the LD_{50} of CVE was expected to exceed 3000 mg/kg body weight.

Change in body weight

There were no significant changes (*P<0.05*) in body weight of the rats in all treatment groups (CVE 500, 1000 and 1500 mg/kg, *p.o*, n=6) compared to the control (Figure 1) after 14 days.

CVE effects on hematological and biochemical parameters

Oral administration of CVE (500, 1000, or 1500 mg/kg) daily for fourteen days did not cause any drug-related changes in the blood parameters (Table 2[A]). There were no significant variations *(P>0.05)* noticed among the hematological parameters of CVE when compared to the control (Table 2[A]) accordingly. Furthermore, the results obtained in the biochemical analysis did not show any significant differences *(P>0.05)* in the CVE (500, 1000, or 1500 mg/ kg)-treated rats compared to the control group, respectively (Table 2[B]).

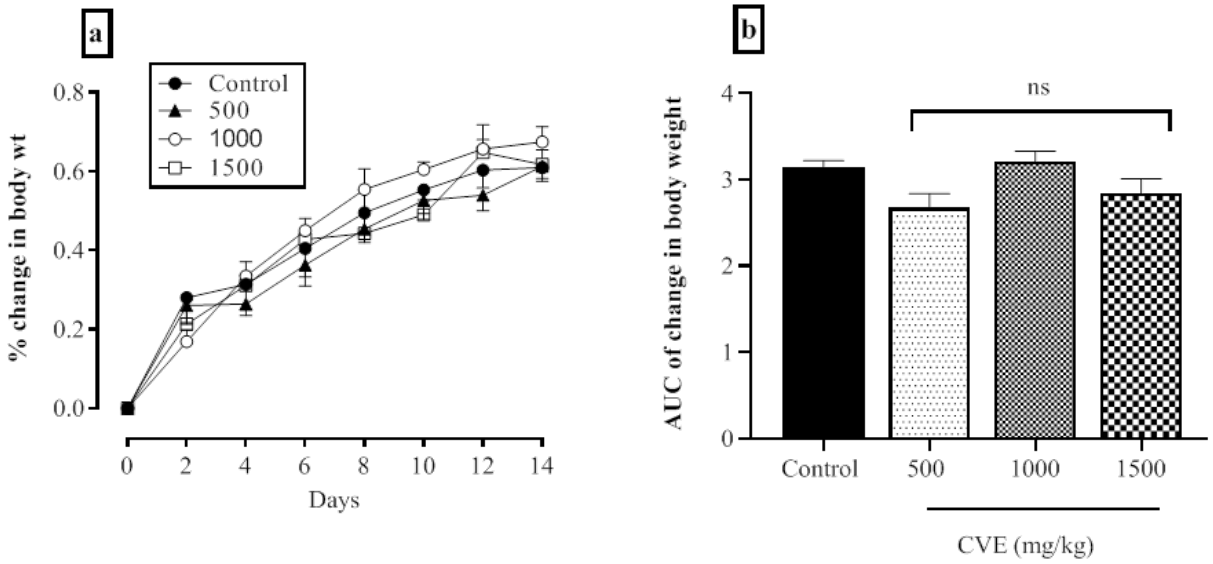


Figure 1: CVE effects on percentage change **(a)** and total body weights **(b)** of rats in acute oral toxicity test. Rats (n=6) received single oral treatment of CVE (500, 1000 or 1500 mg/kg) from day 1 until 14th day. Body weights were taken every other day for 14 days and data was expressed as mean ± SEM. P<0.05 was considered statistically significance in relation to the control.

Table 2A.

CVE impact of hematological indicators in 14-day acute oral toxicity study.

Parameter	Control	500 mg/kg CVE	1000 mg/kg CVE	1500 mg/kg CVE	F-value F (3, 16)	P value
RBC (×10⁶/mm³)	7.04 ± 0.13	6.99 ± 0.23	6.89 ± 0.09	6.99 ± 0.11	0.1611	0.9209
HGB (g/dL)	13.65 ± 0.59	15.10 ± 0.57	14.79 ± 0.45	14.98 ± 0.67	1.354	0.2925
PCV (%)	39.76 ± 0.89	39.85 ± 0.87	40.25 ± 0.66	39.33 ± 1.00	0.0612	0.9795
LUM (×10³/mm³)	4.87 ± 0.45	4.87 ± 0.43	4.92 ± 0.60	4.52 ± 0.54	0.1349	0.9378
WBC (×10³/mm³)	6.71 ± 0. 89	7.54 ± 0. 61	7.91 ± 0.47	6.91 ± 0.65	0.6692	0.5832
PLT (×10³/mm³)	429.9 ± 23.74	448.5 ± 0 24.33	487.50 ± 30.11	434.70 ± 25.17	0.9444	0.4425
MCV (fl)	56.32 ± 0.41	57.07 ± 0.49	57.61 ± 0.27	57.21 ± 0.30	2.018	0.1519
MCH (pg)	18.50 ± 0. 47	19.65 ± 0.40	19.53 ± 0.57	19.15 ± 0. 12	1.4930	0.2544
MCHC (g/dl)	34.89 ± 0. 33	33.02 ± 0.41	33.81 ± 0.58	34.02 ± 0.53	2.656	0.0837
NEU (×10³/mm³)	1.54 ± 0. 20	1.26 ± 0.09	1.12 ± 0.10	1.36 ± 0.15	1.173	0.3511
MON (×10³/mm³)	0.08 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.2821	0.8376
EOS (×10³/mm³)	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.3333	0.8014

Results were expressed as mean ± SEM. P<0.05 was considered statistically significant compared control group.

Table 2B.

Effect of daily oral administration of CVE on biochemical parameters of rats in a 14-day acute oral toxicity study.

Parameter	Control	500 mg/kg CVE	1000 mg/kg CVE	1500 mg/kg CVE	F value F (3, 16)	P value
Total protein (g/dL)	69.51 ± 3.7	62.71 ± 7.82	57.73 ± 9.32	66.23 ± 9.23	0.131	0.7608
Albumin (g/dL)	35.65 ± 2.66	38.72 ± 2.69	33.98 ± 6.43	37.65 ± 8.17	0.968	0.6462
Globulin (g/dL)	34.01 ± 3.72	25.63 ± 4.75	23.83 ± 5.74	28.96 ± 2.84	1.386	0.3276
Albumin-Globulin ratio	1.06 ± 0.05	1.47 ± 0.53	1.57 ± 0.47	1.46 ± 0.34	1.351	0.8407
D- bilirubin(µmol/L)	1.13 ± 0.04	1.27 ± 0.63	1.19 ± 0.28	0.98 ± 0.06	0.964	0.7835
I-bilirubin(µmol/L)	0.58 ± 0.05	0.71 ± 0.08	0.95 ± 0.04	0.74 ± 0.01	2.074	0.3537
Total bilirubin (mg/dL)	1.62 ± 0.07	1.85 ± 0.74	2.03 ± 0.30	1.58 ± 0.07	1.761	0.7753
AST (U/L)	112.76±23.66	103.35 ± 21.32	117.32±17.67	109.43 ± 21.67	1.062	0.4365
ALP (U/L)	342.16±48.77	347.61 ± 53.52	338.34±41.48	346.32 ± 39.84	2.005	0.4673
ALT (U/L)	67.43 ± 75.81	62.45 ± 13.67	69.53±19.54	58.32 ± 12.73	1.652	0.4705
GGT (U/L)	5.93 ± 1.43	4.64 ± 1.08	5.27 ± 1.91	4.83 ± 1.94	1.663	0.3974
BUN (mmol/dL)	5.84 ± 1.76	5.63 ± 1.67	6.17 ± 0.58	5.72 ± 1.07	1.030	0.6532
Creatinine (mmol/dL)	48.34 ± 9.56	49.72 ± 12.45	51.45 ± 10.76	44.72 ± 9.65	1.437	0.4532

Results were expressed as mean ± SEM. P<0.05 was considered statistically significant compared control group.

Effect of CVE on relative organ weight of rats

In this study, there were no significant variations *(P<0.05)* in the relative organ weights of CVE treated rats as compared with those of the control rats (Figure 2).

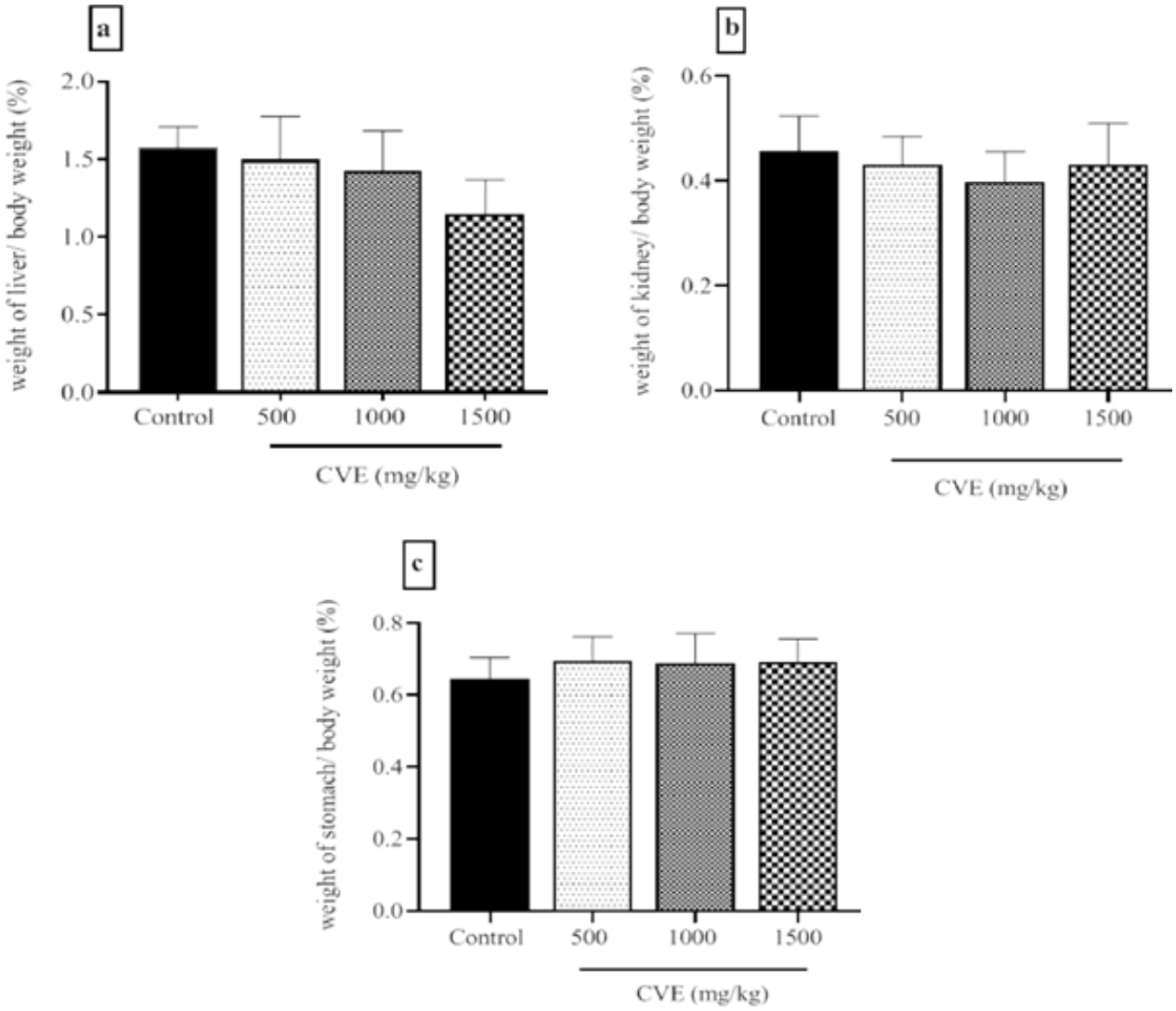


Figure 2: The impact of CVE on the relative organ weights of rats. Rats (n=10) received oral administrations of 500, 1000, or 1500 mg/kg of CVE daily for 14 days. Rats were killed, and the weights of the liver **(a)**, kidney **(b)**, and stomach **(c)** were expressed as a percentage change over the body weights. Results were expressed as the mean ± SEM. P> 0.05 was considered statistically significant.

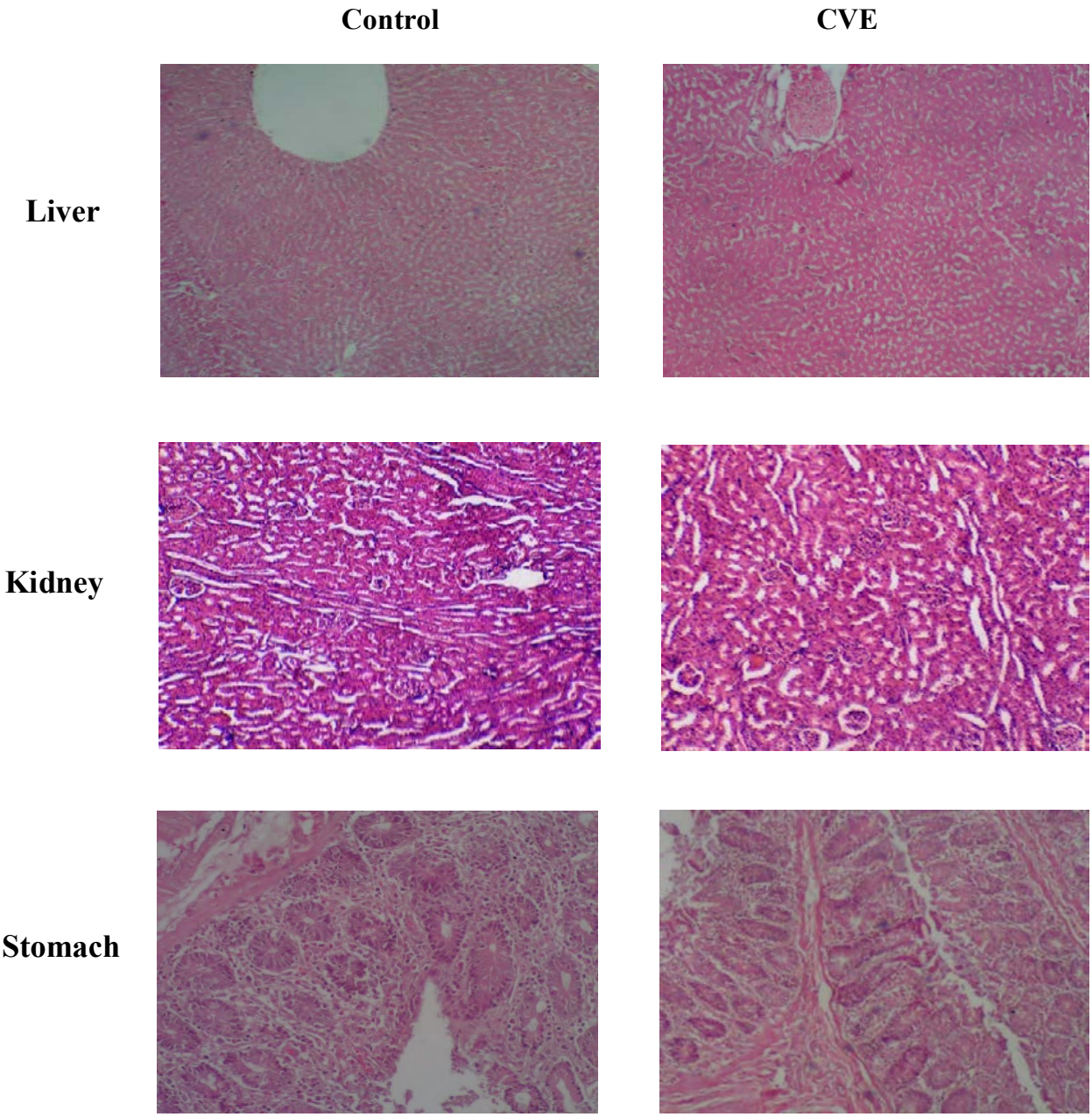
CVE effects on histopathological examination of vital organs

Figure 3 illustrates the histological examination of the vital organs (i.e. liver, kidney and stomach) in CVE-treated rats relative to the control group. Our findings demonstrated the absence of significant hepatocellular injury or dilated sinusoids in the liver. The hepatocytes had a distinct sinusoidal architecture relative to the control group. Nonetheless, the blood vessels inside the portal tract exhibited congestion.

The kidney of CVE-treated rats had a well-defined cortex and medulla, akin to the control group, with renal tubules of uniform dimensions. The glomeruli were intact, exhibiting normal spacing and showed no renal injury in comparison to the control group.

The histological analysis of the stomach of CVE-treated rats revealed preserved epithelium and normal glands in the mucosa, similar to the control group.

Figure 3: Histopathological evaluation of CVE on vital organs in a 14-day acute oral toxicity



study. Vital organs include the liver (blue star= blood vessel; yellow arrows= sinusoids), kidney (yellow arrow= glomerulus), and stomach (yellow arrow= epithelium; blue arrow= glands). Organs were harvested, and tissues were processed. Tissues were sliced into 5µmthick sections and stained with H&E stain. Histological analysis was performed using an electronic microscope. ×100 Magnification.

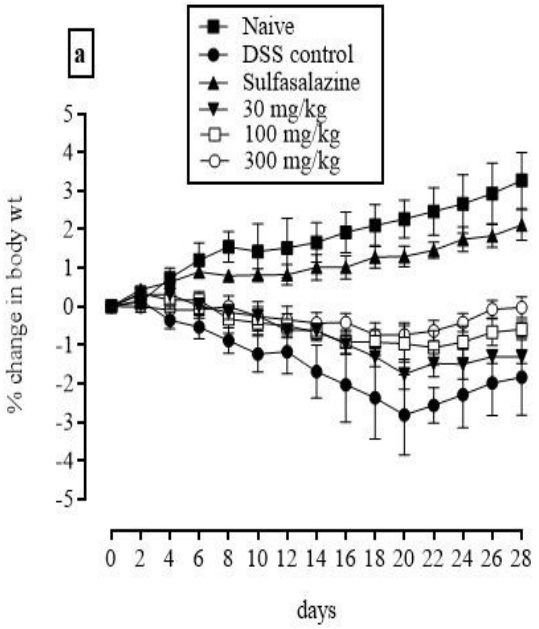
Antiulcerogenic effects of CVE in DSS-induced colitis

Change in body weight

From the study, administration of 5% DSS through drinking water for seven days resulted in reproducible ulcerative colitis characterized by a significant decrease in the mice's gross body weight (Figure 4).

Naive control mice exhibited a modest rise in body weight during the 28-day period. Conversely, the DSS control rats had a steady weight reduction from day 2 (0.35 ± 0.06%) to day 28 (1.87 ± 0.475%), with the peak weight loss recorded on day 20 (2.82 ± 0.07%) [Figure 4a]. The total body weight loss of the DSS control mice (21.73 ± 6.94), as determined by the AUC, exhibited a significant difference *(P<0.001)* when compared to the naive control mice (66.85 ± 12.71) [Figure 4b]. Sulfasalazine (AUC = 56.39 ± 15.42) substantially *(P<0.05)* mitigated DSS-induced weight loss in comparison to the DSS control (AUC = 21.73 ± 6.94) [Figure 4b]. Furthermore, 100 mg/kg CVE (AUC = 41.96 ±

12.55) and 300 mg/kg CVE (AUC = 43.67 ± 13.71) substantially *(P<0.05)* mitigated DSS-induced weight loss in mice relative to the DSS control (Figure 4b) respectively.



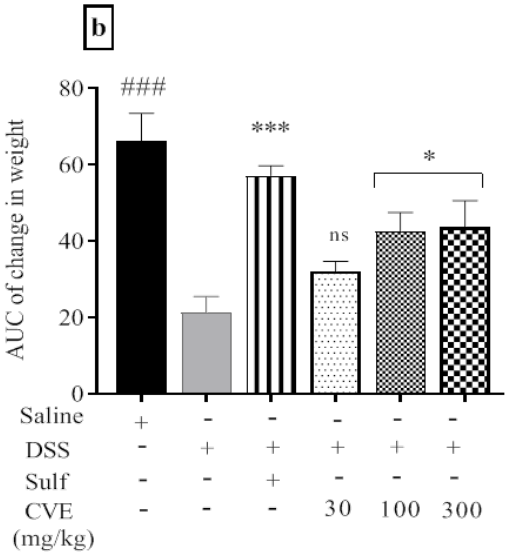
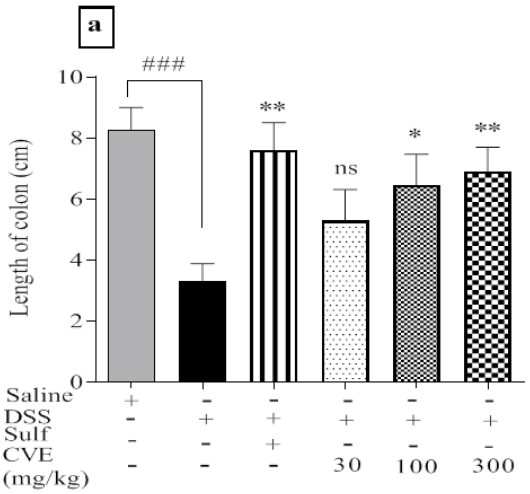


Figure 4: Effect of CVE (30, 100 or 300 mg/kg, *p.o*., n=6) on body weight in DSS-challenged mice. (**a**) and (**b**) represent time-course curves and AUC of body weight change, accordingly. Results were presented as mean ± SEM. *P< 0.05; **P< 0.01; ***P< 0.001; ###*P< 0.001*; ns= non-significant; Sulf= Sulfasalazine. *wt*= weight; DSS= dextran sodium sulphate.

Colon weight and length

The mean colon length of the naive control mice was 8.28 ± 0.72 cm (Figure 5). Administration of DSS significantly shortened the colon length to 3.30 ± 0.58 *(P< 0.001)* compared to naive group. Sulfasalazine significantly increased the colon length to 7.60 ± 0.9028 *(P< 0.01)* compared to the DSS-challenged control (Figure 5a). Similarly, CVE increased the colon length to 6.46 ± 1.01 *(P< 0.05)* and 6.90 ± 0.85 *(P< 0.01)* at doses of 100 and 300 mg/kg, separately (Figure 5a).

The mean colon density of the naive control mice was 23.18 ± 2.128 mg/cm (Figure 5b). DSS significantly increased the mean weight/ length of the colon to 47.88 ± 3.06 *(P< 0.001)* compared to the naïve group. Sulfasalazine significantly reduced the colon weight/ length to 29.98 ± 2.37 *(P < 0.01)* compared to control (Figure 5b). CVE in a similar fashion reduced the mean colon weight/ length ratio to 33.60 ± 3.78 *(P< 0.05)*, 36.92 ± 1.65 *(P< 0.01)* and 31.84 ± 2.98 *(P< 0.001)*, respectively, at doses



of 30, 100, and 300 mg/kg compared to the control group (Figure 5b).

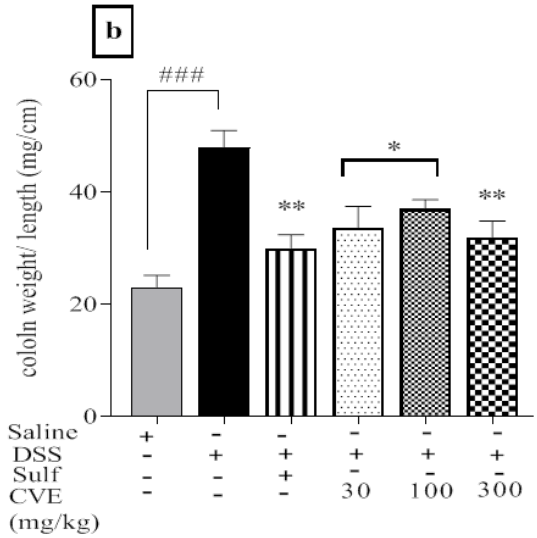


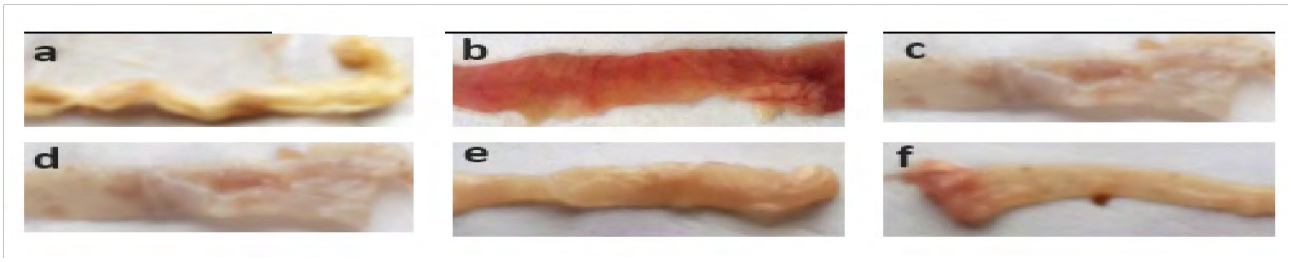
Figure 5: Effect of CVE on the colon length (**a**), colon weight/ length ratio (**b**) and wet/ dry fecal weight (**c**) in DSS-challenged mice. Results were presented as mean ± SEM. ###P< 0.001, ##P< 0.01 (naive vs control); *P< 0.05; **P< 0.01 (control vs treatment groups).

Colon Score and disease activity index (DAI)

In this study, colons of DSS-control mice exhibited excessive hyperemia, edema, ulceration, and thickening of the intestinal wall of the mucosa (3.66 ± 0.16) [Figure 6A[b], B). However, there were no observable changes in the colons of the naive control mice (Figure 6A[a], B). Sulfasalazine treatment significantly

(P< 0.01) prevented severe ulcerative lesions in the colons attaining a colon score of 1.46 ± 0.19 (Figure 6A[c], B). Similarly, CVE-treated mice exhibited a significant reduction of colonic lesions, erythema, edema, and erosion of the mucosal surface (Figure 6A [d,e,f]). Colon scores for CVE-treated mice were 2.62 ± 0.27 *(P< 0.05)*, 2.34 ± 0.29 *(P< 0.05)*, and 2.20 ± 0.17 *(P< 0.05)* at doses of 30, 100, and 300 mg/ kg, respectively (Figure 6A, B).

The DAI scored on the factors of behavior and appearance, such as rectal bleeding, raised fur, immobility, hunch posture, feeding, and mortality are shown in Table 3 (Kishi et al., 2022). In this study, CVE recorded lower DAI scores compared to the DSS-challenged control group (Table 3)



B

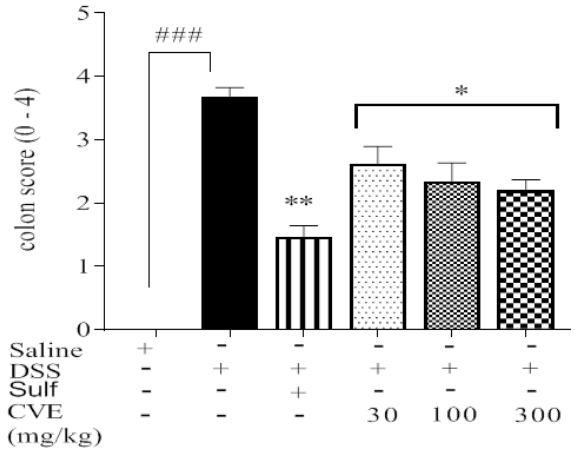


Figure 6: Effect of CVE on the colon score in DSS-challenged mice (n=6). Colonic injuries such as edema, hyperemia, erythema, thickening, and shortening were scored on a 0-4 scale. Photographs show colons of mice treated with **A** [(**a**) distilled water only; (**b**) DSS + saline; (**c**) DSS + sulfasalazine; (**d**) DSS + 30 mg/kg CVE; (**e**) DSS + 100 mg/kg CVE; (**f**) DSS + 300 mg/ kg CVE]. Scores were presented as the mean ± SEM. ###P< 0.001 (naive vs. control); *P< 0.05; **P< 0.01 (control vs. treatment group).

Table 3.

Disease activity index of mice observed from day 0 to day 28.

Treatment	Rectal Bleeding	Diarrhea	Raised fur	Immobility	Hunch posture	Food Intake	Mortality
Naive	0	0	0	0	0	regular	0/6
Control	1	2	2	3	5	irregular	1/6
Sulfasalazine	0	0	1	0	2	regular	0/6
CVE 30 mg/kg	1	2	2	2	4	regular	0/6
100 mg/kg	0	2	1	1	3	regular	0/6
300 mg/kg	0	1	1	1	3	regular	0/6

Histopathological evaluation of CVE-treated colon tissues

According to the study, histological investigations of the colon tissues of naive mice did not show any sign of inflammation (Figure 7A). The DSS-challenged control mice had severe colonic inflammation characterized by epithelial degradation, disruption of the intestinal glands, and necrosis (Figure 7B). Tissues of sulfasalazine-treated mice exhibited significantly reduced epithelial damage with almost intact intestinal glands (Figure 7C). The CVE-treated groups similarly demonstrated a substantial decrease in the destruction of the epithelium and inhibited disruption of the Intestinal glands (Figure 7[D-F]).

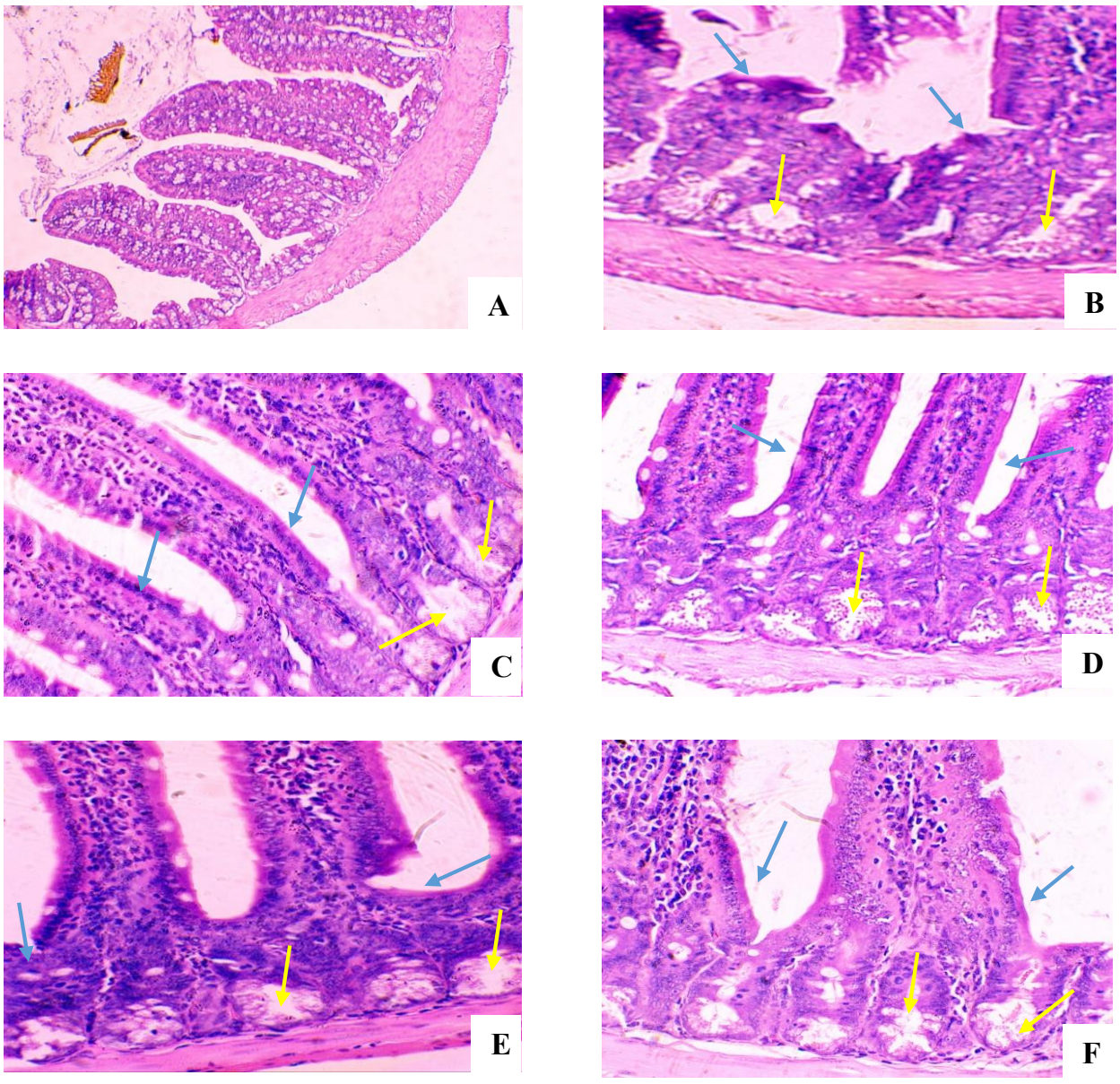


Figure 7: Photomicrographs of colons of mice (×40 magnification). Mice were treated with: (A) distilled water only; (B) DSS + saline; (C) DSS + sulfasalazine; (D) DSS + 30 mg kg^{-1} CVE; (E) DSS + 100 mg kg^{-1} CVE; (F) DSS + 300 mg kg^{-1} CVE. Blue arrows indicate epithelial layer; Yellow arrows denote intestinal glands.

CVE effects on hematological parameters

In this study, the administration of DSS for 7 days significantly *(P< 0.05)* reduced the RBC, HGB, and HCT levels and increased WBC levels. Treatment of mice with sulfasalazine significantly *(P< 0.05)* increased the RBC, HGB, and HCT levels compared to the control.

Comparatively, CVE markedly elevated *(P< 0.05)* the levels of RBC, HGB, and HCT relative to the control group. Levels of WBC, LYM, and PLT for the naive and experimental groups were not statistically different (Table 4).

In vivo antioxidant activity of CVE

Administration of DSS is associated with oxidative stress. Myeloperoxidase (MPO), malondialdehyde (MDA), and glutathione (GSH) concentrations were used to evaluate oxidative stress in the colons. The naive control mice exhibited mean levels of 37.12 ± 8.70 nmol/mg protein for MPO, 67.65 ± 0.57 nmol/mg protein for MDA, and 201.56 ± 11.70 µmol/mg protein for GSH. The colons of the DSS-treated mice exhibited a substantial elevation in MPO and MPA levels, with a reduced concentration of GSH in comparison to the naive control animals. Treatment with sulfasalazine markedly decreased MPO and MDA levels while elevating GSH in comparison to the DSS challenged control group. Similarly, colons of mice treated with CVE exhibited significantly reduced MPO and MDA levels, with elevated GSH levels (Table 5).

Table 4.
Effect of CVE on hematological parameters of DSS-challenged mice.

Group	RBC ($\times10^6$/ mm^3)	HGB (g/dL)	HCT (%)	WBC ($\times10^3$ mm^{-3})	LYM ($\times10^3$ mm^{-3})	PLT ($\times10^3$ mm^{-3})
Naive	6.67 ± 0.38*	14.78 ± 0.18*	36.73 ± 0.27*	2.84 ± 0.16	2.19 ± 0.22	342.63±12.83
Control	3.09 ± 0.45	8.73 ± 0.34	26.78 ± 0.19	3.98 ± 0.44	3.74 ± 0.42	562.43 ± 21.08
Sulfasalazine	6.03 ± 0.28*	14.45 ± 0.39*	35.74 ± 0.31	2.15 ± 0.78	2.73 ± 0.16	468.72±18.06
30 mg/kg CVE	4.96 ± 0.31	12.93 ± 0.07	33.83 ± 0.77	3.03 ± 0.55	2.38 ± 0.88	474.83 ± 51.05
100 mg/kg CVE	5.73 ± 052	13.72 ± 0.25*	35.32 ± 0.34	2.72 ± 0.17	2.36 ± 0.09	422.03 ± 71.03
300 mg/kg CVE	5.95 ± 0.22*	14. 67± 0.18*	34.92 ± 0.39	1.54 ± 0.18*	2.29 ± 0.53	391.23 ± 21.46

Results were presented as mean ± SEM. *P< 0.05 compared with control group.

Table 5.
Effect of CVE on MPO, MDA and GSH levels in DSS-challenged mice.

Treatment	MPO (nmol/mg protein)	MDA (nmol/mg protein)	GSH (µmol/mg protein)
Naive	37.12 ± 8.70###	67.65± 0.57#	201.56 ± 11.70#
Control	201.38 ± 31.20	277.19± 0.71	141.76 ± 14.76
Sulfasalazine	63.92 ± 4.80**	102.34 ±0.76*	194.67 ± 12.97*
30 mg/kg CVE	187.64 ± 28.62	193.76 ±0.87*	146.67 ± 11.80
100 mg/kg CVE	149.33 ± 17.32*	170.57 ± 0.34*	173.56 ± 0.57*
300 mg/kg CVE	157.71 ± 10.21*	163.82 ± 0.79*	173.87 ± 0.95*

Results were presented as mean ± SEM and statistically compared with DSS control. #P< 0.05, ###P< 0.001 (naive vs control); *P< 0.05, **P< 0.01, ***P< 0.001 (control vs treatment group).

DISCUSSION

In this study, we examined the acute oral safety and antiulcerogenic potential of *Cordia vignei* leaf extract in chronic DSS-induced ulcerative colitis. This work represents, to our knowledge, the first assessment of the toxicity of *Cordia vignei* leaf extract and the evaluation of its therapeutic effects in chronic inflammation. The relevance of natural products in the treatment of several pathological conditions cannot be overemphasized. Numerous studies conducted have proven that plants possess phytomedicinal properties with therapeutically potent phytochemical constituents attributed to their medicinal activities. Alongside the establishment of novel phytomedicinal effects and the assessment of effectiveness of natural products such as plants, the safety profile of plant extracts is very important (Chandrashekar et al., 2022).

Acute toxicity investigation, which entails the determination of LD_{50}, is essential for conducting toxicological assessments of substances especially plant extracts (Nalimu et al., 2022). This research mainly evaluates mortality, behavioral modifications, body weights, and other spontaneous alterations in the overall well-being of rats after single and continues exposure of the extract for 14 days. From the study, the acute toxicity test showed that the ethanolic leaf extract of *Cordia vignei*, given to rats at a dose of up to 3000 mg/kg, did not cause any mortality, behavioral, or physiological abnormalities in the rats within 14 days of administration. Thus, the LD_{50} of the extract was estimated to be above 3000 mg/kg, which is consistent with the Hodge and Sterner classification (Hodge & Sterner, 1949) categorizing the *Cordia vignei* leaf extract as relatively non-toxic.

The assessment of the body weight of experimental animals is crucial in determining the toxicity and safety of a natural substance. In addition, the relative organ weight is vital for assessing whether an organ has sustained harm or not (Nalimu et al., 2022). Moreover, a decrease in body and organ weights serves as an indicator of toxicity (Nalimu et al., 2022). In this study, CVE did not significantly alter the body and relative organ weights of the rats, which indicates that the extract is relatively safe.

Hematological and biochemical indicators serve as fundamental diagnostic requirements in medical care, primarily to assess the toxicity of compounds (Murwanti et al., 2023; Kauser et al., 2023). These clinical parameters are employed to define, identify, and describe the harmful effects of compounds after administration in humans or animals (Murwanti et al., 2023). The analyses are relevant in determining the specific target organ of the plant extract, and provide essential insights into its potential to induce blood or organ toxicity. Our findings revealed that CVE caused no significant alterations in the hematological and biochemical markers. There were no substantial changes in the indicators of both blood and biochemical analyses in the CVE-treated rats compared to the control group. These findings suggest that CVE is relatively non-toxic with no associated toxic effects in neither blood cells nor biochemical components.

Histopathological evaluations of organs can offer significant insights into the impact of a test compound on their microscopic structures, contributing to the safety and toxicity assessment of medicinal plants (Abebe, 2023). This assessment aims to examine abnormalities in organ pathology (Murwanti et al., 2023). Our findings in this study revealed that the gross examination of the organs (i.e. liver, kidney, and stomach) revealed no evidence of necropsy or aberrant morphological anomalies. Therefore, no significant histoarchitectural alterations were noticed in the organs of CVE-treated group when compared to the control group. There were no substantial microscopic changes in the liver, kidney and stomach after 14 days of CVE administration. Hence, these findings suggest that the administration of CVE at doses up to 1500 mg/kg.bw once daily for 14 repeated days may not cause any extract-induced organ toxicity. Consequently, the histological findings of CVE evidently support the hematological and biochemical analyses. However, further toxicity assessments using subacute or chronic studies utilizing repeated doses of CVE, are necessary to validate its safety for prolonged use.

DSS concept is a recognized experimental framework that closely mimics the first signs of human inflammatory bowel disease (IBD), including weight loss, diarrhea, hematochezia, mucosal ulcers, colonic atrophy, and injury to colonic epithelial cells (Joshi et al., 2024). This model is essential for understanding the pathophysiology of IBD and evaluating prospective treatment approaches. The integrity of the intestinal barrier, maintained by intestinal epithelial cells, is essential for distinguishing the intestinal lumen from the internal environment. This barrier is essential for safeguarding intestinal tissue from dangerous microorganisms, poisons, and other detrimental chemicals while also facilitating appropriate immune activity (Thoo et al., 2019). The disruption of this barrier is a characteristic feature of IBD and greatly contributes to the course of the illness (Chung et al., 2024; Guo et al., 2022). Drinking DSS water for a prolonged period provokes chronic inflammation akin to human in IBD (Joshi et al., 2024). Administration of DSS (5%) led to weight loss, diarrhea, anorexia, and the presence of blood in stool, which are key characteristics of the disease potentially arising from mucosal injury and impaired epithelial barrier function (Joshi et al.,

2024). However, CVE therapy marginally increased the body weight of the rats at 100 and 300 mg/kg, and improved the colon length at the same doses. Furthermore, the colon weight-to-length ratio was elevated substantially at all doses. Hence, the decrease in colon weight-to-length ratio by CVE suggests its anti-inflammatory activity, particularly in reducing the enhanced influx of cell infiltration and edema, which are hallmarks of increased colon weight relative to tissue length (Joshi et al., 2024).

Elevated Disease Activity Index (DAI) is a defining characteristic of DSS-induced colitis (Guo et al., 2022; Alqudah et al., 2024). The DSS-challenged control group exhibited higher DAI scores, but CVE treatment significantly reduced these scores. This downregulation of DAI scores by CVE suggests its ameliorative effects in ulcerative colitis therapy.

Histopathological alterations are usually associated with IBD disorders (Govindarasu et al., 2024). The histological alterations observed in DSS-challenged control mice consist of significant epithelial damage, degradation of intestinal glandular cells, necrosis, edema, reduction of goblet cells, and escalated infiltration of inflammatory cells (Govindarasu et al., 2024). Treatment with CVE, however, decreased destruction of the intestinal glands, fibrosis and prevented enhanced erosion of the epithelial cells as well as the mucosal layer. Thus, the inhibitory effects of CVE against histopathological changes during colitis makes it a good alternative and complementary source for treating ulcerative colitis.

In IBD, hematological distortions are prominent, which are markers of anemia and infection. In this study, the DSS-challenged control mice exhibited significant alterations in hematological parameters, characterized by increased WBC and decreased RBC and HGB levels. Therapy with CVE restored the hematological variations in the DSS-treated mice indicating its significant role in combating inflammation during ulcerative colitis (Joshi et al., 2024).

Oxidative stress and inflammation are related phenomena. Intense inflammation induces the generation of free radicals, thereby disrupting the body's redox balance (Wang et al., 2022). The overabundance of reactive oxygen species (ROS) may result in oxidative damage to intracellular lipids, proteins, and DNA, ultimately compromising the integrity of the intestinal barrier (Li et al., 2023). Oxidative stress is a significant factor in the manifestation of IBD (Tatiya-aphiradee et al., 2020). Consequently, mitigating oxidative stress and maintaining redox balance represents a potential therapeutic approach for ulcerative colitis (Li et al., 2023). This study demonstrated that the administration of 5% DSS in mice resulted in increased levels of MPO and MDA, alongside a decrease in GSH levels. However, treatment with CVE in our study improved oxidative stress by upregulating the levels of GSH and lowering the MPO and MDA levels caused by DSS-challenged oxidative injury in the colon. This indicates that CVE may contribute to the mitigation of oxidative damage and inflammation, thereby potentially reducing disease severity (Tahvilian et al., 2021). Therefore, the phytomedicinal activities of CVE in attenuating oxidative stress and colonic inflammation are attributed to the phytochemical constituents present in the plant (Owusu et al., 2023)

CONCLUSION

The current work has shown that acute oral toxicity assessments of *Cordia vignei* leaf extract (CVE) resulted in no mortality or harmful effects at any dosage, with an estimated LD_{50} above 3000 mg/kg of body weight. Furthermore, CVE did not cause any hematological, histological or biochemical changes, indicating that it may be relatively safe. The antiulcerogenic properties of CVE were exhibited by lowering oxidative stress, reducing ulcerative colitis, and maintaining mucosal integrity.

Limitation of the study

This study is limited by the lack of isolation of specific chemical compounds from *Cordia vignei* by our team that mediate its antiulcerogenic effects. Furthermore, the possible mechanisms of action through which it exhibits the antiulcerogenic properties needs to be investigated.

Acknowledgement

We acknowledge the enormous assistance from the technicians in the Department of Pharmacology, KNUST, Kumasi, Ghana and the entire research assistances in the other institutions for the success of this research.

Author's contributions

MAA and **GO** were responsible for the conceptualization, study design, sample collection and extraction, methodology, data analysis and interpretation, writing, critical revision and proofreading of the manuscript.

BA, FKA, and WAA were responsible for data collection, data analysis and interpretation, critical revision and proofreading of the manuscript.

RAA was responsible for sample collection and extraction, proofreading of the manuscript. All authors read, revised, and approved the manuscript.

Ethical Approval

All protocols for the study were approved by the ethics committee of the Department of Pharmacology, KNUST, Ghana (Ref: ECCOL/2017/021).

Conflict of interest

Authors declare that there is no conflict of interest.

Funding

The study was funded privately by the individual members of the research team.

Data Availability

The data used for this study will be available upon reasonable request from the corresponding author.

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