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IMMUNOPATHOLOGICAL EVALUATION OF GREEN SYNTHESIZED (ZINC OXIDE NANOPARTICLE OF AQUEOUS FRACTA) EXTRACT AS A TREATMENT OF ULCERATIVE COLITIS IN RATS

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ABSTRACT

Interest in exploring natural substances as alternative treatments for bowel diseases continues to grow. Although green synthesis has repeatedly been mentioned in stimulating both arms of the immune system, this has led to the attraction of inflammatory cells for healing colitis. This study aimed to assess the impact of green-synthesized Zinc Oxide nanoparticles and aqueous extract on enhancing immune and pathological defense mechanisms against colitis in rats. Sixty adult male rats were categorized into six groups at random, (G1) Acetic acid (4%) (1.5 ml/intrarectally), (G2) normal saline, (G3) *Prosopis farcta* hydrochloric extract (400 mg/kg/orally), (G4) ZnO-NP of *Prosopis farcta* (400 mg/kg/orally), (G5) ZnO-NP of *Prosopis farcta* 400 mg/kg/ orally, in combination with sulfasalazine 100 mg/Kg/ orally) and (G6) sulfasalazine group. At the end of the experimental period (20 days), serum was taken for inflammatory markers (TNF-α, IL-1β and IL-6 levels), and the colon tissues were examined histologically. Results showed elevated serum levels of immunological biomarkers between treated and untreated groups. These indicators were observed in the (G1) group. The green synthesized group showed reductions in pro-inflammatory cytokines TNF-α (15.148), IL1β (4.02) and IL-6 (7.88), compared with (G1) (79.968), (31.4) and (30.2), respectively. The enhanced immune response related to colon inflammation is improved with these effects, leading to the disappearance of many pathological lesions. This study revealed that the use of green-synthesized supplemented diets, especially in (400 mg/kg), could improve immune responses and protect the colon and reduce symptoms of gastrointestinal diseases, such as ulcerative colitis.

Keywords: Histopathological finding, Immune response, IL 1 β, IL-6, TNF-α

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INTRODUCTION

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), are recurring intestinal diseases characterized by persistent intestinal inflammation (Danese *et al*., 2004). Despite having a chronic history and an unknown cause, UC and CD are similar in that they are both inflammatory conditions. \However, there are some distinctions based on the parts of the gastrointestinal system that are affected and the symptoms that manifest. Minimal colon involvement and persistent mucosal and submucosal inflammation are characteristics of ulcerative colitis (Mosna *et al*., 2021). The increasing demand for nutritious food products and population growth are driving the rapid expansion of the global aquaculture industry. Aquatic protein supplies are essential to this industry since they offer superior, nutrient-dense foods that are good for your health (Shah and Mraz, 2020). However, infectious illnesses have emerged because of intensive aquafarming methods, causing large financial losses (Jeyavani *et al*., 2022). This circumstance also brought attention to pro-, pre-, and symbiotic as well as immunostimulants, such as therapeutic herbs, as a possible source of bioactive compounds and substitutes for the use of antibiotics (Essawi and Srour 2000). Active molecules derived from plants, known as phytochemicals, have anti-stress, immuno-stimulant, biocidal, and growth-promoting properties (Wang *et al*. 2009). Steroids, alkaloids, flavonoids, terpenoids, phenolics, and essential oils are the primary categories into which plant-derived chemicals fall (Doan *et al*. 2020; Hoseinifar *et al*. 2020a). Herbal immunostimulants are also good alternatives to chemical components in diets because of their many positive benefits (Van doan *et al*. 2019). The abundance of a variety of secondary metabolites in plants gives them the ability to treat and/or prevent diseases, which have also been shown to be effective against certain bacteria and fungi (Stratev *et al*. 2018). The industrialized nanotechnology science today is capable of producing novel nanoparticles. The odd thing is that nanoparticles (NPs) have already participated in the production of a wide range of lucrative applications in many dynamic fields, such as agriculture, industry, and medicine. They can also pass through any biological membrane because of their extremely small size, which ranges from 1 to 100 nm. According to the research, NPs have been employed in a variety of commercial imposts at the bazaar; yet, they have been shown to induce inflammatory responses and oxidative stress (George *et al*., 2018; Durazzo *et al*., 2020). One of the most advised biological techniques for the manufacture of nanoparticles is green synthesis. Using plants that have natural reducing agents like flavonoids to create nanoparticles (Myriam *et al*., 2012). The vital trace elements that are involved in many processes, such as nutrition metabolism, bone growth, and development. Zinc's mediating action on T-lymphocytes shown that it is necessary for the immune system to operate normally (Prasad and Elumalai, 2011). This study aimed to assess the anti-inflammatory and immunostimulatory properties of green produced zinc oxide nanoparticles of *Prosopis farcta* extraction in reducing colitis in the rat model.

MATERIALS AND METHODS

Preparation of *Prosopis farcta* herbal using Soxhlet extraction.

According to Direkv and-moghadam and Ghasemi-seyed, (2014) *Prosopis farcta* herbal was collected from Tikrit city, herbal sellers (Apothecary) 2024. The plant was detected by an expert (botanical) from the Department of Seed Authentication in the Ministry of Agriculture of Iraq. Using Soxhlet extraction, *Prosopis farcta's* bioactive components was extracted by drying the fruit at a low temperature (less than 50°C), grinding it into a fine powder, and weighing 100–150 g of the powder.

The powder was put in a Soxhlet thimble or filter paper inside the extractor chamber. Depending on the target compounds, we chose distilled water as the appropriate solvent and added 250–500 mL to a round-bottom flask. To make the solvent appear clear in the siphon tube, the Soxhlet apparatus was assembled heat the solvent to enable repeated cycles of vaporization, condensation, and extraction, and then the operation was repeated for 6–12 hours. Once cooled, the crude extract was concentrated by evaporating any remaining solvent and the solvent was recovered using a rotary evaporator or distillation. The extract was stored at 4 °C for additional analysis. For use in later processes, this technique (Fig.1) guarantees the effective separation of bioactive substances.

Fig. 2. *Prosopis farcta* extract

Synthesis of ZnO nanoparticles. . to make a 0.1 M zinc precursor solution by dissolving zinc acetate dihydrate (2.19 g in 100 mL distilled water) and a 0.2 M NaOH solution (0.8 g in 100 mL distilled water) to create ZnO nanoparticles using a chemical precipitation approach according to (Umar *et al*., 2018). Gradually the NaOH solution was added dropwise while keeping the temperature constant and stirring vigorously after heating the zinc solution to 60 to 70°C.

Preparation of green-synthesized Pro-ZnO NPs. For the biosynthesis, 500 milliliters of deionized (DI) water were used to dissolve 35 grams of the dried *Prosopis farcta* extract. An equal mass (35 g) of purified ZnO nanoparticles was added to the solution. The mixture was stirred continuously using a magnetic stirrer to ensure uniform dispersion. Sodium hydroxide (NaOH, 0.1 M) was gradually added to adjust the pH to 12, facilitating the reduction of Zn^{2+} ions and the precipitation of ZnO nanoparticles. For five hours, the reaction mixture was continuously stirred and kept at 80°C. Following the reaction, the resulting suspension was dried at 90 °C. The dried material was then subjected to calcination at three different temperatures 500 °C, 600 °C, and 700 °C, each for 2 hours, in order to determine the optimal calcination conditions for nanoparticle formation. The final product was a bright brownish powder, identified as ZnO nanoparticles synthesized using *Prosopis farcta* extract, with a milky appearance before calcination (Miri *et al*., 2020).

Characterization of synthesized green (Pro-ZnO NPs).

The description was achieved using monochromatized CuKα1 radiation at a wavelength of (λ = 1.5406 Å), the Bruker D2-Phaser X-ray diffractometer was able to determine the crystal structure of each manufactured zinc oxide sample. Using the Thermo Nicolet IR 200 (USA), IR spectroscopy was carried out. TA Instruments USA's Universal V4.5A differential scanning calorimeter was used for DSC-TGA. The FEI Nova 450 Nano SEM field emission scanning electron microscope was used to examine surface morphology. The FE-SEM device was run at 10 kV of accelerating voltage. UV and Fourier-transform infrared (FTIR; range 4000–400 cm−1) tests were performed on the synthesized NPs (Alamdari *et al*., 2020). Analysis was exerted to study the crystalline nature and purity of synthesized ZnO-NP (Alrubaie and Kadhim, 2019).

Rat husbandry and experimental design.

All national and international guidelines for the care and use of animals (ethical approval) in research were adhered to in this study (local approval no.: Tu. Vet. 73 12/11/2024).

Induced colitis for pilot study.
Before starting the investigation, we carried out a pilot study to show that acetic acid can induce colitis. Nine rats were given varying concentrations of acetic acid in the rectum, with doses of 1.5 ml, 3%, 4%, and 5% alternately (Cinpolat *et al*., 2023), then the animals were euthanized after five days. The 4% acetic acid dose demonstrated its capacity to induce colitis, and an efficient dose for inducing inflammation and assessing immunity.

Study design. 60 male albino rats, 250-300 mg/kg were divided randomly into 6 groups. Each group contained (10) rats divided as follows:
G1, the control positive treated with acetic acid- 4% 1.5 ml intrarectally for 4 days.
G2, the control negative, given normal saline rectally.
G3, treated with *Prosopis farcta* hydrochloric extract at a dose of 400mg/Kg B.W orally for 15 days.
G4 treated with ZnO – NP of *Prosopis farcta* (extract) 400 mg/Kg B.W orally for 15 days.
G5 treated with ZnO-NP of *Prosopis farcta* extract, 400 mg/kg B.W, in combination with sulfasalazine 100 mg/Kg B.W) orally for 15 days.
G6 treated with sulfasalazine 100 mg/kg orally for 15 days. This study was performed in the Wet Laboratory of the Lab Animals Housing, Faculty of Veterinary Medicine, Tikrit University, Iraq.

Study plan.
Step1: Preparing male rats for treatment of colitis after fasting the rats for 24 hours to induced colitis, then by using feeding tube which introduced 3 cm proximal from the anus in the Trendelenburg position, groups (G 1, 3, 4, 5 and 6) were given 1.5 milliliters of 4% ethanoic acid under light sedation with chloroform for 4 days and watching them daily for the clinical signs of colitis (weight loss, diarrhea, gross rectal bleeding after that treating them. Group 1 received normal saline.

Step 2: All the study groups except G2 group were given the previously stated medication according to their group protocol daily from 0 to 15 days through gastric gavage.

Step 3: On day 20, blood was taken from all groups of study and serum was obtained and kept at − 80 °C for estimation of the levels of inflammatory markers (TNF-α, IL-1β and IL-6 levels). Then, the rats were euthanized by cervical dislocation, and the colon samples were collected for macroscopic and histopathological examination.

Evaluation of immunological biomarkers.
Rats' pro-inflammatory cytokines TNF-α, IL-1β 1β and IL-6 evaluation. The concentration of IL-6, IL-1β and TNF-α pro-inflammatory cytokines in the rat serum samples was measured using Enzyme-Linked Immunosorbent Assay (ELISA) Kits (SUNLONG Co. LTD) based on the manufacturer's instructions. The standard and samples were pipetted into wells containing rat IL-6, IL-1β and TNF-α specific immobilized antibodies, and they were thereafter incubated at 37 °C for 30 minutes. After washing and incubation, a horseradish peroxidase-conjugated streptavidin was pipetted into each well, then incubated at 37 °C for 30 minutes. Chromogens A and B were introduced to the wells, incubated for 15 minutes at 37 °C to produce a certain color, and then measured at 450 nm. The findings were reported as (pg/mg protein) (Tazawa *et al*., 2019).

Macroscopic and microscopic examination.
On day 20 following the last treatments, tissue samples from the treated and control groups were gathered for histopathological analysis. Tissues were fixed in 10% buffered formalin, dried in a series of alcohol grades, cleaned in xylene, and then embedded in paraffin wax. For histological investigations, many 5 µm slices of each

block were produced and stained with hematoxylin and eosin (H&E) (Spencer *et al*., 2012).

Statistical analysis. IBM SPSS software (version 23.0; IBM Corp., Armonk, NY, USA) was used for statistical analysis and means ± SE were used to present the results. Data analysis was done for multiple comparisons using the post hoc test (LSD) after one-way analysis of variance (ANOVA). The significance threshold was set at $P<0.05$.

RESULTS

Characterization of Pro-ZnO NPs

Potential stability of Pro-ZnO NPs

In order to investigate the morphology of the synthesized ZnO-N of the extract *Prosopis farcta*, FESEM images were recorded through the FESEM microscope (MIRA3 TESCAN, Czech Republic) (Fig. 2A). Overall, Fig. (2B) revealed an aggregation of a high number of ZnO nanoparticles in the prepared samples and the particle size distribution of ZnO of *Prosopis farcta* extract samples. This graph was obtained by examining the diameter of 100 different nanoparticles from different points and was evaluated with Digimizer image processing software and finally, the results were placed on the graph. The average diameter of Zn-O-N-P.P.E. was 30.725 nm and the low standard deviation of 4.292% indicates homogenous and high-quality synthesis of these one-nanostructures.

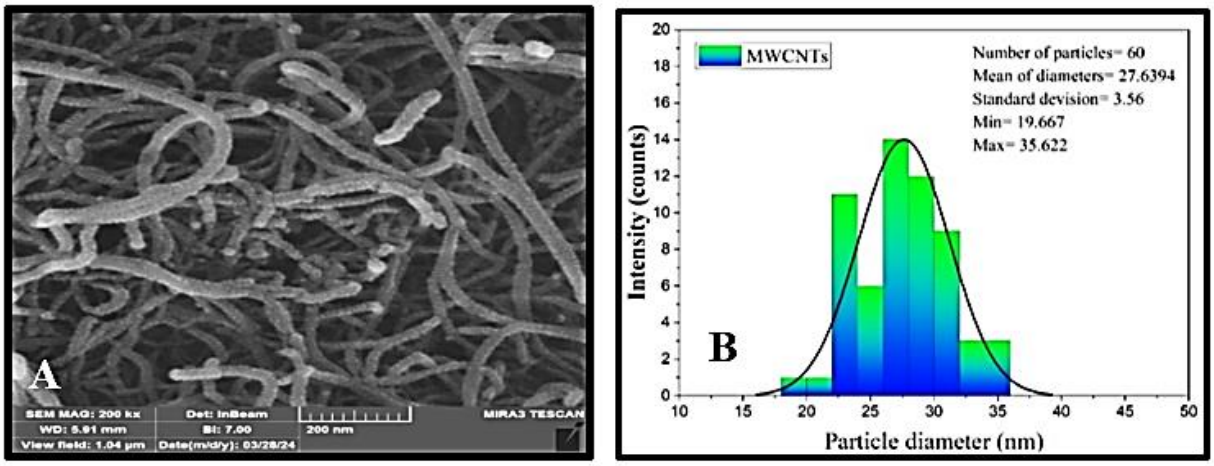

Fig. 2. The findings of nanoparticle observations utilizing (**A**) FESEM image of MWCNTs at the scale bar of 200 nm, (**B**) PSD curve of MWCNTs.

X-ray Diffraction (XRD) interpretation of Pro-ZnO NPs.

The powder X-ray diffraction (XRD) analysis (Fig. 3C) was recorded through the X-ray diffractometer with Cu Ka radiation (wavelength: 0.15406 nm) at a counting time of 20 minutes and a step size of 0.05°. The qualitative analysis of this sample revealed that the Zinc oxide nanoparticle of the extract of *Prosopis farcta* fruit pattern completely corresponds to the hexagonal phase of zinc oxide with space group of P63mc and JCPDS number of (01-079-02-07). The main diffraction angles of ZnO nanostructure are located at 32.02°, 34.80°, 36.61°, 47.94°, 56.91°, 63.27°,66.75°, 68.19°, 69.46°,72.90°, and 77.26°, which belong to (100), (022), (101), (102), (110), (103), (200), (112), (201), (004), and (202) reflections. The bending vibration of the C-O-H phenol groups is responsible for the absorption band at 1406 cm-1 in Fig. (3D). The strong adsorption band at 1608 cm-1 is attributed to the stretching vibration of c=c aromatic rings in the extraction sample. The broad peak in 3372 cm-1 is related to the stretching vibration of OH groups that surround the ZnO nanoparticles.

Assessment of immunological and biochemical biomarkers

The pro-inflammatory cytokines in the current study analysis using post hoc intergroup comparison testing showed a significant increase (P<0.005) in serum concentration of TNF-α, IL-1β and IL-6 in the untreated acetic acid-induced colitis group (control positive), compared to the control negative and treated groups (based on distribution of data) (Figs. 4A, B and C). Additionally, there were no discernible changes in the level of TNF-α between the EXP group (17.374±0.956), and the N.S group (17.254±0.898). While the serum concen-tration of IL-1β showed a significant increase in G6 (6.232±0.725), compared to G3 and G4 (4.858±0.721and 4.020±0.548). The current analysis data of IL-6 serum level represented no significant change between treated groups (G3, 4 and 5) (8.494 ± 0.633, 7.882±0.322, and 8.686±0.450, respectively. All the treatment groups effectively reversed the changes in these characteristics when compared pairwise, with the exception of the group with the control negative group.

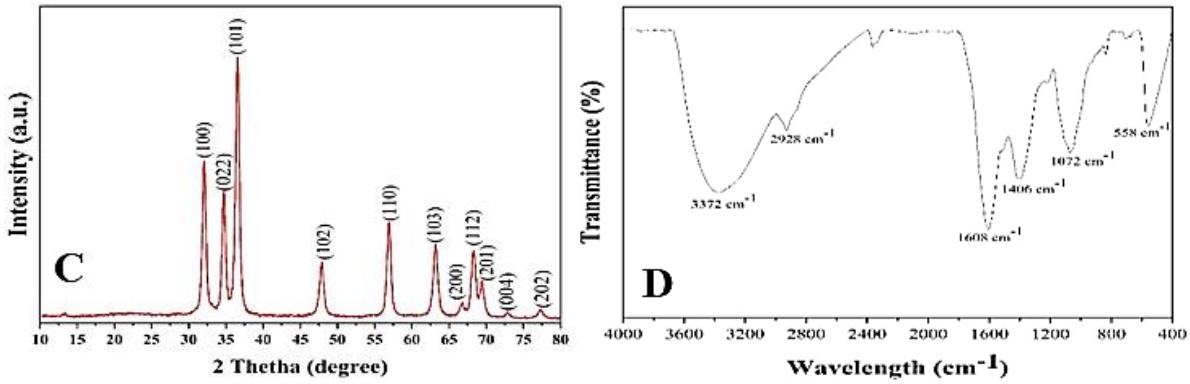

Fig. 3. (**C**) X-ray diffractograms of samples of Pro-ZnO NPs. (**D**) The Pro-ZnO NPs sample's FTIR spectrum.

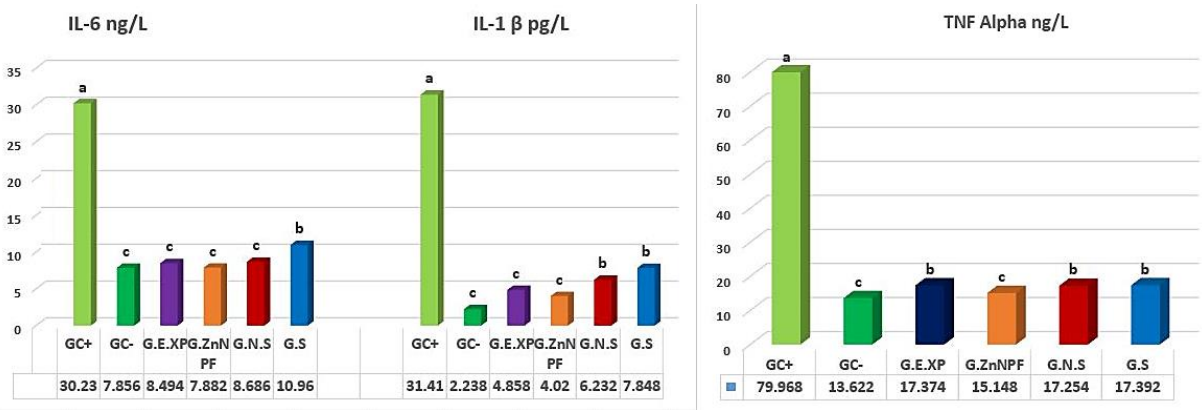

Fig. 4. (**A**) and (**B**) represent the previously established statistically significant variations in serum levels of several inflammatory markers, including IL-6 (ng/L) and IL-1β (pg/L), in treated and non-treated groups at 20 days post-treatment. (**C**) Serum levels of TNF-α (ng /L) in treated and non-treated groups at 20 days post-treatment. The same letters indicated no differences between them, *Significant difference (ANOVA, P<0.05) between control and treatment groups.

Histopathological colonic findings

Tissue section of colon in G1 revealed severe damage of villi, which was characterized by severe pseudomemb-ranous hemorrhagic colitis with fibrinous exudate, necrosis with depletion of tubular glands, inflammatory infiltrates with severe mucosal hemorrhage and submucosal congestion (Fig. 5A). Section of G2 colon of showed normal appearance of epithelium

mucosa, simple tubular gland, normal cellular loose connective tissue of lamina propria and submucosa (Fig. 5B). Colon of G3 showed normal mucosal lining cells with mild hyperplasia of lining cells of tubular glands (Fig. 5C). Colon of G 4 showed normal mucosal lining cells with mild hyperplasia of lining cells of tubular glands & vascular congestion (Fig. 5D). However, G5colon showed normal mucosal lining cells with atrophy of tubular glands and infiltration of lymphocytes and vascular congestion (Fig. 5E). In addition, G6colon showed normal mucosal lining cells with marked subepithelial thickening associated with inflammatory infiltrate and severe mucosal submucosal vascular congestion with little atrophy of tubular glands (Fig. 5F).

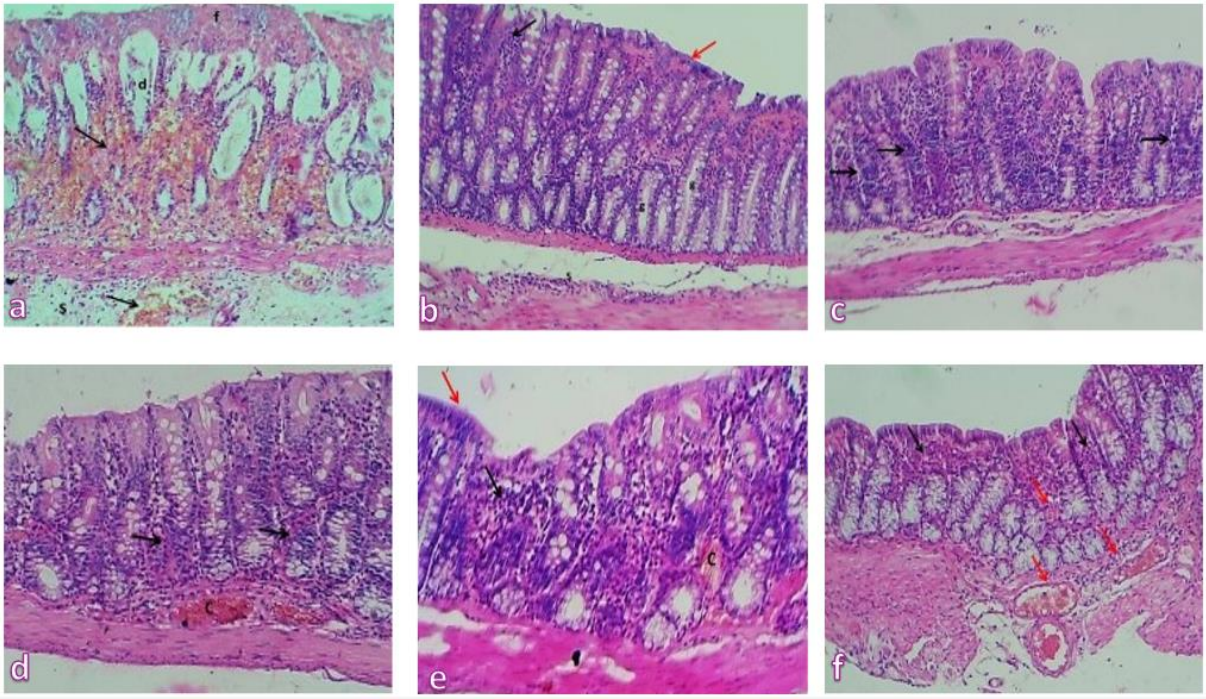

Fig. 5. Micrograph of the histopathological structure of colon tissue in different groups at the end of the treatments stained with H&E (100x): **A)** (G1) shows severe pseudomembranous hemorrhagic colitis, with fibrinous exudate in colon (f), necrosis with depletion of tubular glands (d), inflammatory infiltrates with severe mucosal hemorrhage (black arrows) & submucosal congestion. **B)** (G2) shows normal appearance of the epithelium mucosa in colon (Red arrow), simple tubular gland (g), normal cellular loose connective tissue of lamina propria (black arrow) & submucosa (S). **C)** (G3) shows normal mucosal lining cells with mild hyperplasia of lining cells of tubular glands (Black arrows). **D)** (G4) shows normal mucosal lining cells with mild hyperplasia of lining cells of tubular glands (Black arrows) & vascular congestion (C). **E)** (G5) shows normal mucosal lining cells (red arrow) with atrophy of tubular glands and infiltration of lymphocytes (black arrows) & vascular congestion. **F)** (G6) showing normal mucosal lining cells with marked subepithelial thickening associated with inflammatory infiltrate (Black arrows) & severe mucosal submucosal vascular congestion (red arrows) with little atrophy of tubular glands (g).

DISCUSSION

One of the inflammatory, recurring, and chronic intestinal diseases is ulcerative colitis (UC). Although its exact cause is uncertain, a number of studies have shown that the pathophysiology of colitis is influenced by genetic, environmental, and altered immune variables. Despite the increased awareness in recent years of that illness, the treatments used are still inadequate (Nagib *et al*., 2013). Acetic acid was utilized in this study to compare

experimental chemical colitis models with the control and treatment groups. Pro-inflammatory cytokine levels and histological results were measured to assess the effectiveness of the experimental groups (Fig. 4). The increase in pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) levels in serum was strongly correlated with ulcerative colitis and inflammation (Banerjee *et al*., 2019). In the early stages of inflammation, lymphocytes and macrophages release tumor necrosis factor-α (TNF-α), a vital pro-inflammatory cytokine that can also trigger several mediators that end inflammation. It has been observed that TNF-α has a significant role in the pathophysiology of colonic ulcers (Xiao *et al*., 2016). The elevation of serum concentration of pro-inflammatory cytokines is commonly known in ulcerative colitis, which as an inflammatory illness involves multiple inflammatory pathways, including NF-κB, STATs, and MAPK, and is marked by significant pro-inflammatory responses (Li, *et al*., 2021). Before delving into the interpretation of the results of our current study to explain the variation in the level of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) levels, it must be taken into consideration that the zinc nanoparticle was taken at a low concentration. Zinc nanoparticle was currently considered a vehicle for the *Prosopis farcta* extract used in the experiment. Moreover, current results differ from many previous studies that used zinc at higher concentrations. Our data demonstrated that Pro-ZnO NPs and treated groups suppress both NF-κB-induced pro-inflammatory cytokines and COX-2-induced prostaglandin synthesis that cause inflammation. These findings come to Zn+2 mediated reduction of TNF-α, IL-1β, and IL-6 release is consistent with earlier research in the LPS-induced endotoxemia paradigm (Klostedudfen and Hauptmann, 1996). Another study demonstrated that when 4 μg/mL ZnO NPs were administered to HepG2 cells, IL-6 and IL-1β are dose-dependently downregulated (Agarwal and Shanmugam, 2020). Additionally, it has been demonstrated that ZnO NPs prevent human lymphocytes from releasing TNF-α in response to acetylcholinesterase inhibitor chlorpyrifos, which causes inflammation and cytotoxicity (El Sheikh *et al*., 2024). The difference in concentrations of zinc nanoparticles used in this experiment may not be consistent with previous studies that indicated the ability of nanoparticles to stimulate the secretion of cytokines (Al-Bayati and Alwan, 2020; Al-Bayati *et al*., 2024). Mucosal and submucosal inflammation was observed in the acetic acid-induced colitis model. Necrosis, vascular dilatation, swelling and other symptoms are indicative of inflammation. Histopathologically, the inflammation brought on by acetic acid in the colon was similar to UC (Randhawa *et al*., 2014). The co-administration of green syntheses to treated groups resulted in modest lymphoid depletion, necrotic cell regeneration, and colon structure amendment. According to our microscopic results, it might restrict the immune response and antioxidant content of this substance. Because it can restore inflammatory indicators, it can therefore heal and lessen colonic degeneration. Likely, Zamudio-Cuevas *et al*. (2021) found that the herb inhibited the nuclear factor-κB-dependent pathway in the injured cells following toxin treatment, hence reducing the release of proinflammatory cytokines.

CONCLUSION

Our data concluded that either alone or mixed treatments of green synthesized Zinc Oxide NPs of aqueous extract of *Prosopis farcta* may reduce pro-inflammatory cytokine production of TNF-α, IL-1β and IL-6.

Conflict of interest declaration

Regarding the research, writing, and/or publication of this paper, the authors have disclosed no potential conflicts of interest.

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التقييم المناعي المرضي للمستخلص الأخضر المُصنّع (جسيمات أكسيد الزنك النانوية من الفراكتا المائية) كعلاج لالتهاب القولون التقرحي في الجرذان

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يتزايد الاهتمام باستخدام المواد الطبيعية كعلاجات بديلة لأمراض الأمعاء، وقد تم الإشارة مرارًا إلى دور التخليق الأخضر في تحفيز كلا فرعي الجهاز المناعي، مما يؤدي إلى جذب الخلايا الالتهابية للمساهمة في شفاء التهاب القولون. هدف هذه الدراسة هو تقييم تأثير الجسيمات النانوية لأكسيد الزنك المُخلقة بطريقة خضراء والمستخلص المائي النباتي للفراكتا المائية على تعزيز الآليات الدفاعية المناعية والمرضية ضد التهاب القولون في الجرذان. تم تقسيم ستين جرذًا ذكريا بالغًا عشوائيًا إلى ست مجموعات: المجموعة الأولى (G1): تم تحفيز التهاب القولون باستخدام حمض الأسيتيك بتركيز ٤٪ وبجرعة ١,٥ مل داخل المستقيم لمدة ٤ ايام وبالمثل تم اعطاء باقي المجموعات, باستثناء المجموعة الثانية (G2): تم إعطاؤها محلول ملحي طبيعي لتمثل المجموعة الضابطة السالبة. المجموعة الثالثة (G3): تم علاجها بمستخلص نبات *Prosopis farcta* المائي بجرعة ٤٠٠ ملغم/كغم عن طريق الفم. المجموعة الرابعة (G4): عُولجت بجسيمات أكسيد الزنك النانوية المُخلقة من *Prosopis farcta* بجرعة ٤٠٠ ملغم/كغم عن طريق الفم. المجموعة الخامسة (G5): عولجت بنفس الجسيمات النانوية بجرعة ٤٠٠ ملغم/كغم بالإضافة إلى دواء السلفاسالازين بجرعة ١٠٠ ملغم/كغم عن طريق الفم. المجموعة السادسة (G6): مجموعة السلفاسالازين فقط. في نهاية الفترة التجريبية (٢٠ يومًا)، تم جمع عينات الدم لقياس المؤشرات المناعية (TNF-α، IL-1β وIL-6)، كما تم فحص أنسجة القولون مجهريا. أظهرت النتائج ارتفاع مستويات المؤشرات المناعية في المصل بين المجموعات المعالجة وغير المعالجة، وكانت هذه المؤشرات مرتفعة بشكل واضح في المجموعة الأولى (G1). بينما أظهرت مجموعة المعالجة بالجسيمات النانوية المُخلقة خفضًا ملحوظًا في السيتوكينات الالتهابية IL-6 (4.02) IL-1β (15.148) TNF-α (٧,٨٨) مقارنة مع المجموعة (G1) التي أظهرت مستويات (٧٩,٩٦٨) ، (٣١,٤) ، و(٣٠,٢) على التوالي. وقد أدى هذا التأثير إلى تحسين الاستجابة المناعية المصاحبة لالتهاب القولون، مما ساعد على اختفاء العديد من الآفات المرضية. أظهرت الدراسة أن استخدام الأنظمة الغذائية المدعمة بالجسيمات النانوية المُخلقة خضرًيا، وخاصة عند جرعة ٤٠٠ ملغم/كغم، يمكن أن يُحسن الاستجابات المناعية ويوفر حماية للقولون ويقلل من أعراض الأمراض المعوية مثل التهاب القولون التقرحي

الكلمات المفتاحية: النتائج النسيجية المرضية، الاستجابة المناعية، انترلوكين ١ بيتا، انتر لوكين ٦, عامل نخر الورم ألفا