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Aloe vera gel attenuates acetic acid-induced ulcerative colitis in adult male Wistar rats

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ABSTRACT

Ulcerative colitis is a disease of undetermined etiology and treatment. It affects the colon and rectum and typically involves the mucosa, manifesting as continuous areas of inflammation and ulceration. Aloe gel contains more than a hundred potentially active constituents of different classes. This study investigated the effect of aloe gel on experimentally-induced ulcerative colitis. Male Wistar rats were randomly allocated into groups A to F of six rats each. Ulcerative colitis was induced to rats in groups B to F by single intra-colonic administration of 2 mL of 4% acetic acid with a size 6F pediatrics catheter. In contrast, group A received an equivalent volume of normal saline by the same route. Twenty-four hours after induction, rats in groups B and C received normal saline and 1 mg/kg b. wt. daily dose of dexamethasone, respectively. In contrast, those in groups D, E, and F received 20, 40, and 60 mg/kg b. wt. doses of aloe gel, respectively, for 14 days. They were sacrificed 24 h after the last administration. We assessed disease progression by determining the clinical activity index, gross inflammation, histological alterations, the intensity of DNA in colon cells, and tissue level of nitric oxide. All the parameters but one increased significantly in group B rats. The quantitative distribution of DNA in colon cells reduced significantly in this group. Aloe gel doses significantly reversed these changes in a dose-dependent manner. Dexamethasone showed lesser efficacy relative to 60 mg dose of the Aloe gel extract. We conclude that Aloe vera gel has therapeutic potential in the treatment and management of ulcerative colitis. The most significant effects were observed in the groups treated with the highest dose of Aloe gel (60 mg/kg b. wt.). It is also worth noting that the remediated potential of aloe gel in acetic acid-induced UC surpasses that of dexamethasone.

1. Introduction

Ulcerative colitis (UC) is a disorder characterized by a continuous lesion within the colon [1]. It occurs any time in life and has a pathognomonic sign of inflammatory process, extending proximally from the rectum and confined to the mucosa [2]. Patients with UC are 10–20 times more likely to develop colorectal cancer [3]. Although its actual cause is undetermined, it is believed to be related to environmental, genetic and abnormal reactions of the immune system [2,4]. Dealing with UC symptoms can seriously affect patients' overall quality of life [5,6].

Some of the conventional treatments for UC have been largely ineffective, while the somewhat effective ones are costly and are associated with significant side effects [7,8]. The ultimate goal for UC treatment is complete remission; thus, whether a phytonutrient-rich plant with reported antioxidant and anti-inflammatory properties would be beneficial to UC treatment remains a progressive study that requires continual optimization.

Aloe vera gel is a viscous and transparent liquid extracted from the parenchymatous cells in the fresh leave of Aloe vera [9]. It contains biologically active constituents like vitamins which function as antioxidants and neutralizers of free radicals [10]. Aloe gel is also rich in steroids such as cholesterol, campesterol, β-sitosterol, and lupeol, all of which have anti-inflammatory action [11]. The mineral constituents of the gel, such as calcium, selenium, magnesium, sodium, and zinc, are potent antioxidants [11,12]. The gel also contains alkaline phosphatase, catalase, the biological enzymes which help to reduce excessive inflammation [9,11]. The hormonal components of the gel are auxins and gibberellins; they facilitate wound healing and have anti-inflammatory action [13]. Considering the varieties of biologically

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active compounds it possesses, we investigated the effects of aloe gel extract on experimentally–induced UC.

2. Materials and methods

2.1. Chemicals and drugs

Acetic acid (identification number: 102200) was procured from Chad Well Health, England. Dexamethasone tablet (Batch number 181001) was supplied by Afochef pharmaceuticals Ado Ekiti, Ekiti State, Nigeria. Nitric Oxide was manually assayed for in the Biochemistry Laboratory of Obafemi Awolowo University (OAU), Ile-Ife.

2.2. Preparation of extract

Mature and healthy Aloe-Vera plants were harvested from Botanical Garden, OAU, Ile-Ife, Nigeria. A taxonomist in the Department of Pharmacognosy identified the plant and a voucher specimen (REFNO: FPI 223) was deposited in the herbarium for reference. The plants were washed in water, and the thick epidermis was peeled off. The gel was then scooped with a spatula and blended in a blender. Homogenate was concentrated and freeze-dried with a lyophilizer. The yield was stored in a desiccator before use.

2.3. Rats care and management

Thirty-six male Wistar rats (120 and 150 g) were procured from the College of Health Sciences animal house OAU. We housed them in plastic cages under standard laboratory conditions of temperature, humidity and light. They had free access to standard laboratory rat chow (Ace feed, Osogbo, Nigeria) and water. Ethical clearance (IPHOAU/12/1366) was obtained from Health Research and Ethics Committee (HREC) of Institute of Public Health, OAU, Ile-Ife, Nigeria. They received humane care according to the guidelines for the use of experimental animals.

2.4. Experimental design

The rats were randomly assigned into six groups (A to F) of six rats ($n = 6$) each. Colonic lesion was induced following overnight fast in groups B, C, D, E, and F by single intra-colonic administration of 2 mL of 4% acetic acid/kg b.w with pediatrics catheter (size 6F) [14]. The catheter was inserted through the rectum up to a distance of 8 cm. Rats in group A received an equivalent volume of normal saline by the same route. Twenty-four hours later, groups D, E, and F received 20, 40, and 60 mg/kg b. wt. oral doses of aloe gel extract, respectively for 14 days [15]. Group B received an equivalent volume of normal saline, while group C received dexamethasone (1 mg/kg b. wt. daily), dissolved in normal saline, for the same period [16]. Animals were sacrificed 24 h after the last administration.

2.5. Assessment of UC clinical activity

Rats were observed on days 1, 8, and 16 post induction/onset of treatment for a composite of weight loss, rectal bleeding and stool consistency.

Weight loss was determined on scales 0–4. 0 for no weight loss, 1 for weight loss of 1–5%; 2 for weight loss of 5–10%; 3 for weight loss of 10–15%, and 4 for weight loss of $>$ 15% following measurement on a top loader digital balance.

To assess stool characteristics, rats were assigned a score of 0 for normal stool; 2 for loose stool, and 4 for diarrhea.

Rectal bleeding was assessed using scores 0–4. 0 for no bleeding (normal stool), 1 for minimal streaks of blood seen with stools, 2 for maximum streaks of blood seen with stools for half of the time, 3 for obvious blood with stool most of the time and 4 for the passage of blood alone, respectively.

The scores were summed and divided by 3, forming a total clinical score that ranged from 0.0–4.0 in which 4 was considered the worst possible situation and 0 as the best.

2.6. Surgical excision of colon

The rats were sacrificed by cervical dislocation 24 h after the last administration. Colons were excised, weighed, washed in normal saline and the distal portions of the colons were inspected for gross alterations. Diagnostically critical information observed were recorded while being processed for further macro and microscopic examination, biochemical assay. After macroscopic examination, one portion was fixed in 10% neutral buffered formalin for histological & histochemical studies while the other was frozen at $-$ 20 °C for biochemical assay for nitric oxide.

2.7. Macroscopic assessments

The distal portions of the colon were carefully opened longitudinally, washed in physiological saline to remove fecal residues. Gross inflammation scores were assigned based on the following morphological criteria: Intact epithelium was assigned a score of 1, patchy type superficial hyperemia as 2, generalized patchy type hyperemic region was given a score of 3, and generalized hyperemia and Hemorrhage was assigned 4.

2.8. Histological procedures

The fixed colon tissues were processed for paraffin embedding. Sections of 5 μm thickness were produced on rotary microtome (Leica RM 2125, RTS), stained with hematoxylin and eosin for general tissue morphology investigation, Verhoeff – van Gieson stain for elastic and collagen fibers demonstrations.

2.9. Histochemical procedures

Fixed colon tissues were processed for routine paraffin embedding and stained for Feulgen reaction to demonstrate DNA localization.

2.10. Microscopic assessment

The level of mucosal inflammation and healing was assessed histologically by using the criteria described by Noronha-Blob et al. [17]. 0 for intact epithelium, no leukocytes or hemorrhage; 1 for $<$ 25% disrupted epithelium, focal leukocyte infiltrates, and focal hemorrhage; 2 for 25% disrupted epithelium, focal leukocyte infiltrates, and focal hemorrhage; 3 for $\leq$ 50% disrupted epithelium, wide spread leukocytes, and hemorrhage; 4 for $>$ 50% disrupted epithelium, extensive leukocyte infiltration, and hemorrhage.

2.11. Estimation of nitric oxide level

The frozen sections were homogenized in 10 mM Tris-HCl buffer, the homogenates were centrifuged at 3000 rpm for 10 min at room temperature, and the level of nitric oxide was determined spectrophotometrically in the supernatant in accordance to the methods described by Ridnour et al., [18].

2.12. Photomicrography and image analysis

Distal colon sections were examined under LEICA research microscope (DM750) connected to a digital camera (LEICA ICC50 - HD), and permanent photomicrographs were taken at the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife, Nigeria. Representative micrographs were uploaded to the Fiji image J analysis software, and scale was set using a digital micrometer gauge reading to

convert measurements in pixels to microns. Nuclear DNA intensity was determined on Feulgen stained micrographs (medium power, 400x).

2.13. Statistical analysis

Statistical Package for Social Science (SPSS) was employed for analyses. Results were evaluated by one-way analysis of variance followed by Duncan post hoc tests for multiple comparisons. Data were expressed as Mean ± SEM and were considered significant at $p < 0.05$.

3. Results

3.1. Highlight the mechanism of action by which Aloe gel attenuates acetic acid induced ulcerative colitis in rats

3.1.1. Effect of aloe gel extract on clinical activity index

Disease activity was significantly higher in the group administered with acetic acid only (2.17 ± 0.09) compared with the normal control (0.00 ± 0.00) as presented in Fig. 1. Aloe gel at all doses administered significantly reduced this index from the worst observed activity seen in group B to close to no activity in groups D, E and F (0.77 ± 0.28; 0.72 ± 0.39; 0.28 ± 0.11 respectively). 60 mg/kg b. wt. of aloe gel reduced clinical activity index better than dexamethasone (0.28 ± 0.11 and 0.39 ± 0.05 respectively).

3.2. Effect of aloe gel extract on gross morphology of the colon

Acetic acid caused a significant increase in gross inflammation score when compared with the normal control group. Aloe gel at all doses administered dose-dependently reversed the inflammatory changes when compared with the negative control group. Dexamethasone had a comparable effect with the 40 mg dose of the extract (Table 1; Fig. 2).

3.3. Histological assessment and scoring

As shown in Table 2, there was an increase in crypt lesions scores in group B (administered with acetic acid only) compared with normal control. All doses of aloe gel reduced these changes when compared with negative control. Their efficacy was comparable with the reduction observed in dexamethasone-treated rats.

3.4. Effect of aloe gel extract on colon histoarchitecture

As shown in Fig. 3, normal colonic mucosa defined by intact crypts arrangements and span was observed in normal control rats. Crypt epithelium, lumina, cellularity (goblet cells and colonocytes) were also preserved in this group. Deranged mucosa features such as disrupted crypts architecture, reduced goblet cell population, sloughed surface epithelium, non-even distribution of crypts were observed in negative control group. Rats treated with 20, 40, and 60 mg/kg b. wt. doses of the gel reversed most of these changes in a dose dependent manner.

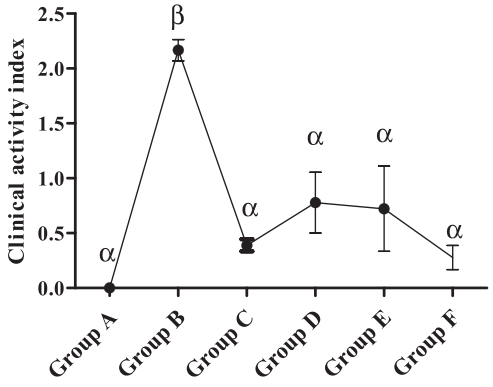

Fig. 1. Clinical activities index score of aloe gel extract treated acetic acid-induced colitis in rats. Values are given as Mean ± SEM in each group. Means with different symbols differs significantly while means with the same symbols do not differ significantly.

Table 1
Gross Morphological Score of aloe gel extract treated acetic acid-induced colitis in rats.

Groups	Mean ± SEM
Group A	1.00 ± 0.00^{a}
Group B	3.17 ± 0.31^{c}
Group C	1.60 ± 0.24^{ab}
Group D	2.33 ± 0.21^{b}
Group E	1.66 ± 0.21^{ab}
Group F	1.33 ± 0.33^{a}

Values are given as Mean ± SEM in each group. Means with different letters differ significantly while means with the same letter do not differ significantly.

3.5. Effect of aloe gel extract on extracellular matrix fibers

As shown in Fig. 4, Verhoeff – van Gieson stain highlight both collagen and elastic fibers. Collagen fibers appeared as band in the lamina propria and the submucosa of the normal control group appeared loose and homogenously distributed. Elastic fiber also appeared uniformly distributed within the lamina propria of this group. Collagen fibers appeared dense in both lamina propria and submucosa in acetic acid-only administered rats, while elastic fibers were clustered in the lamina propria. Collagen fibers were scantily distributed essentially in 20, 40 and 60 mg/kg b. wt. gel-treated rats relative to normal control. Dexamethasone treated rats had comparable distribution to the three treated groups.

3.6. Effect of aloe gel extract on nuclear DNA of colon cells

As shown in Fig. 5, nuclei of colon cells were positive to Feulgen reaction across the groups. Unhydrolyzed control section shows negative reaction to Feulgen stain. Significant reduction in staining intensity was observed in acetic acid only treated group when compared with normal control. Aloe gel significantly increased staining intensity in a dose-dependent manner when compared with untreated group. The increase achieved with 40 and 60 mg/kg b. wt. doses of the gel was similar to the rise seen in the dexamethasone group.

3.7. Effects on aloe gel extract on nitric oxide level

Acetic acid significantly increased tissue level of nitric oxide when compared with normal control rats. All doses of aloe gel significantly reduced NO level in a dose-dependent fashion when compared with negative control. 60 mg/kg b. wt. Aloe vera gel reduces NO level better than dexamethasone (Table 3).

4. Discussion

Ulcerative colitis is an inflammatory bowel disease that affects the mucosal layer of the distal colon and rectum [19]. One of its common symptoms is stool inconsistency, rectal bleeding, weight loss, and pain [20,21]. Bioactive compounds extracted from plants have a long history of use as therapeutic agents [22]. This study was carried out to investigate the efficacy of aloe gel extract against acetic acid-induced ulcerative colitis.

As expected, we observed a significant increase in the disease activity index in acetic acid treated rats. Induction of colitis by acetic acid in rats is one of the reproducible inflammatory bowel disease models [23,24].

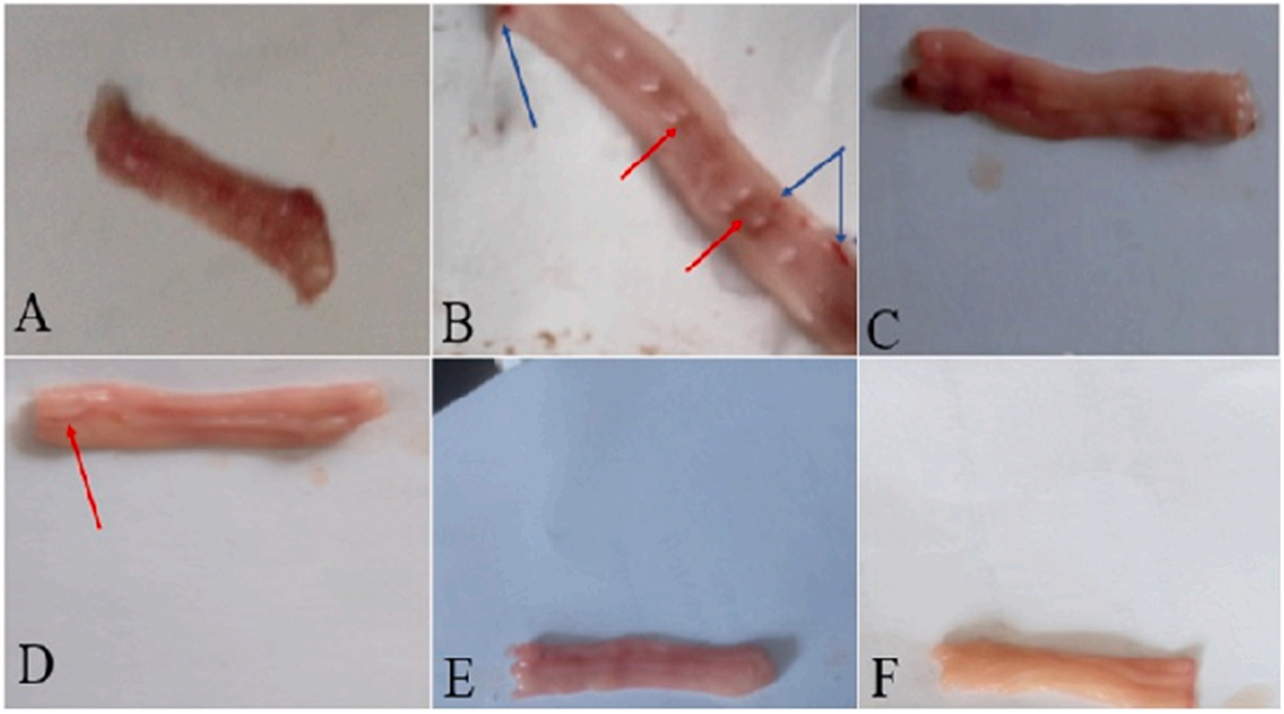

Fig. 2. Representative macroscopic plates of rat colon. Observe (A) Normal colon (B) Gross features of ulcerative colitis with evident thickened /collapsed area (red arrows) and multiple areas of hyperemia (blue arrows). (C, D, E, F) colons appear normal with smallt thickness in its wall in group D. A – control, B – acetic acid + normal saline, C – acetic acid + 0,01 mg/kg b. wt. dexamethasone, D, E, F - acetic acid + 20, 40 and 60 mg/kg b. wt. of Aloe vera gel extract respectively.

Table 2
Histological score of aloe gel extract treated acetic acid-induced colitis in rats.

Group	Mean ± SEM
Group A	0.00 ± 0.00
Group B	1.83 ± 0.30
Group C	0.83 ± 0.30
Group D	1.33 ± 0.33
Group E	0.5 ± 0.22
Group F	0.33 ± 0.21

Values are given as Mean ± SEM in each group

Significant factors contributing to the initiation of human colitis, such as infiltration of inflammatory cells such as neutrophils and increased production of pro-inflammatory mediators are involved in its induction [24]. Other signs and symptoms such as rectal bleeding, stool inconsistency and weight loss have all been reported to be mimicked by this model, which were all validated in this study [25]. Treatment with aloe gel extract caused a significant reduction in disease activity. The exact mechanism of its anti-colitis action might be attributed to its reduction of nitric oxide level in colon tissue, as observed in this study. Nitric oxide has been reported to plays a key role in the pathophysiology of inflammatory process [26]. Flavonol, a type of flavonoid with antioxidant and anti-inflammatory properties was reported to be present in aloe gel, and it is reputed for its potency in inhibiting NO production [27]. Antioxidants selected from a group of vitamins have also been reported to be very effective in treating anorectal disorder. This suggests that antioxidant-rich extract can restore rectal sensitivity, fecal continence and weight gain.

Histological changes associated with UC were significantly reduced by aloe gel treatment. Perturbing a component within the intricate cellular and extracellular networks, as seen in this study is sufficient to disturb tissue homeostasis resulting in functional and morphological disturbances seen in ulcerative colitis. The normal colonic mucosa acts as a barrier against the invasion of potentially harmful substances into the colonic environment and generally in the intestine, thereby preserving the host integrity [28]. In UC, this mucosa barrier is impaired, resulting in a breakdown of the epithelial cells, lamina propria, and subsequent inflammation [29]. The apparent improvement in mucosa health in aloe gel extract groups is indicative of its high medicinal value. It has been reported that fractioned, and the whole unfractionated gel, is rich in antioxidant effects. The gel also is rich in peroxidase activity and phenolic antioxidant [10,11]. Kahramanoğlu et al. [30] reported that approximately thirteen flavonoids including flavone, flavonol and flavan-3-ol have been isolated from aloe gel all of which are potent antioxidant, antiinflammatory and antiulcer agents.

Feulgen reaction remains one of the widely used cytohistochemical reactions in biological and biomedical sciences. It is a nuclear reaction specific for DNA in cytohistochemical samples in situ [31]. The intensity of stain seen in this reaction is consistent with the concentration of DNA. In this study we used Felugen reaction to demonstrate the quantitative distribution of DNA in colon cells at the light microscopic level. We observed a significant decrease in staining intensity of nuclear DNA in UC-treated rats and a significant increase in aloe gel-treated rats. The reduction was thought to be contributed by the infilteration of inflammatory cells into the lamina propria. The inflammatory cells can directly damage the colon tissue and cells and promote the inflammatory response by producing and secreting some cytokines and affect proliferation of cells and activation of T cells, so as to maintain the persistence of inflammatory response [32]. The increase seen in aloe gel treated rats suggests the anti-inflammatory effect of this extract. This was possibly mediated by the steroids present in it such as cholesterol, campesterol, β-sisosterol, and lupeol, all of which possess antioxidant and anti-inflammatory actions [11,30].

In this study, mucosa nitric oxide concentration increased significantly in the negative control group. High concentrations of NO are related to several pathological conditions of the gastrointestinal tract [33]. Active ulcerative colitis is associated with an increase in the activity of the inducible NO synthase [34]. It plays a key role in the pathophysiology of ulcerative colitis by initiating inflammatory process

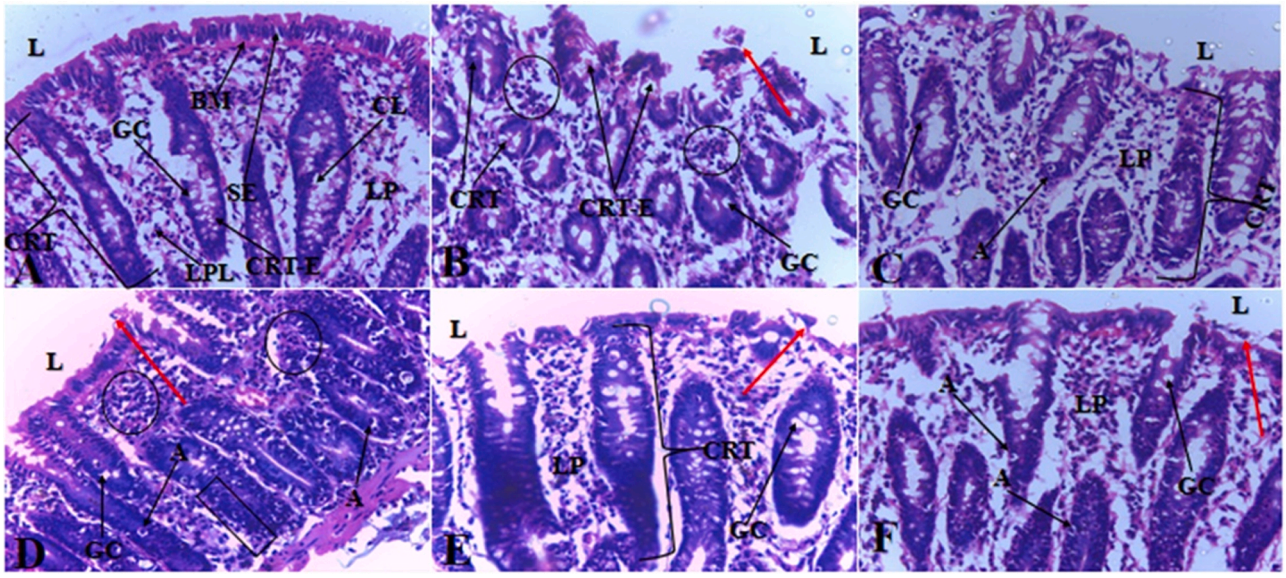

Fig. 3. Representative light micrographs of colon sections of rats subjected to H&E stain. Observe: (A) Colon epithelial integrity (surface – SE and Crypts – CRT-Z) and crypt architecture (brace) are intact. Lamina propria area is evenly divided between cellular elements and intercellular space (LP), prominent subepithelial basement membrane (BM), crypt lumen (CL) and abundant goblet cells (GC). (B) mucosa architectural changes which include marked surface and crypt epithelial injury (CRT-E), sloughed surface epithelium (red arrow), irregular size and shape of crypts (CRT), variety of cells thought of as inflammatory cells in the laminar propria (black circle) and reduced goblet cells population (GC). (C, E, F) no appreciable cryptitis (CRT), some crypts appear short and do not reach the muscularis mucosae (CRT), normal lamina propria cellular elements (LP), mildly sloughed surface epithelium (red arrow), few pyknotic nclei are seen in the crypt epithelium of C and F (A). (D) diffuse inflammatory cells in the lamina propria (black circle), cryptitis (rectangle), reduced goblet cell population, prominent crypt apoptosis (A). (H & E; 400x).

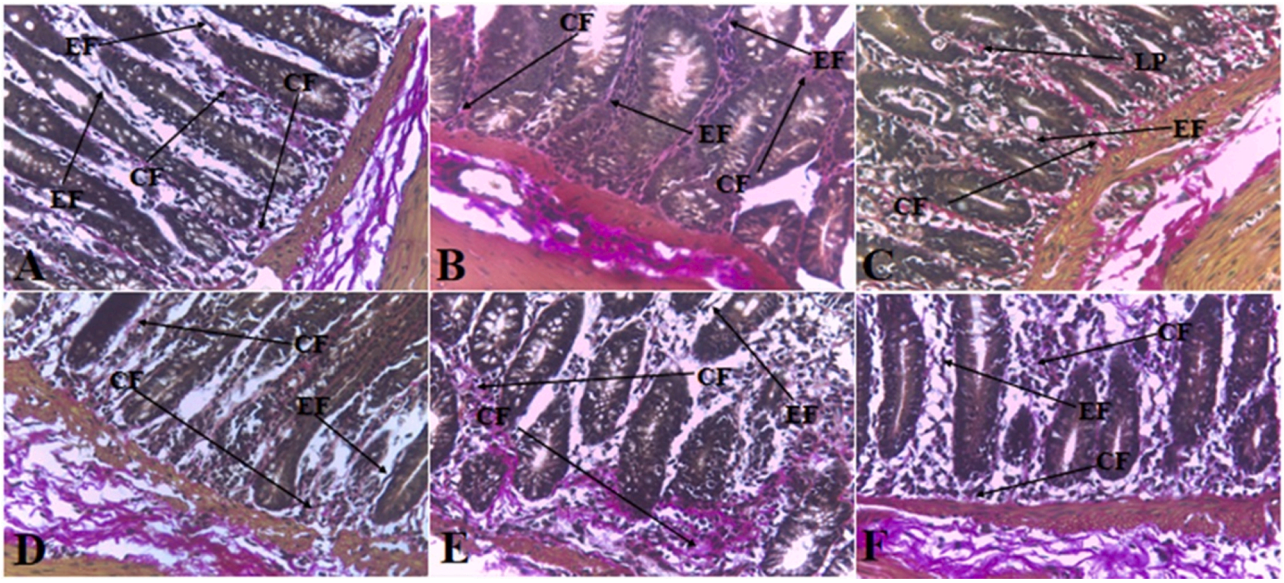

Fig. 4. Representative light micrographs of sections of colon subjected to Verhoeff – van Gieson stain. Observe: (A, C, D, E, F) - Verhoeff's solution highlights of elastic fiber (black) evenly distributed within the lamina propria (CF, EF). Van Gieson's solution highlight (red) of collagen band in the lamina propria (EF) and the submucosa (s). Note the scanty distribution of collagen fibers in these groups. (B) Dense collagen deposits (CF) in both lamina propria and submucosa and heterogenous elastic fibers distributions (EF) in the lamina propria. (Verhoeff – van Gieson stain; 400x).

and perpetuating subsequent damages [35,36]. Liu et al. [37] affirm that oxidative stress occurs when there is accumulation of reactive species which is implicated in the initiation and progress of inflammatory bowel disease. The increase in the NO in the untreated group may have resulted from the accumulation of reactive oxygen specie. However, NO synthesis was significantly reduced in gel extract treated rats. This indicates the anti-inflammatory potential of the extract. In a study that also focuses on the anti-inflammatory potential of aloe gel, it was reported that the anti-inflammatory activities of the Aloe vera gel is due to the presence of C-glucosyl chromone a recently isolated anti-inflammatory compound [38].

5. Conclusion

The improvement in colitis after 2 weeks of Aloe vera covered all the domains of the scoring system. The biochemical assay for NO also

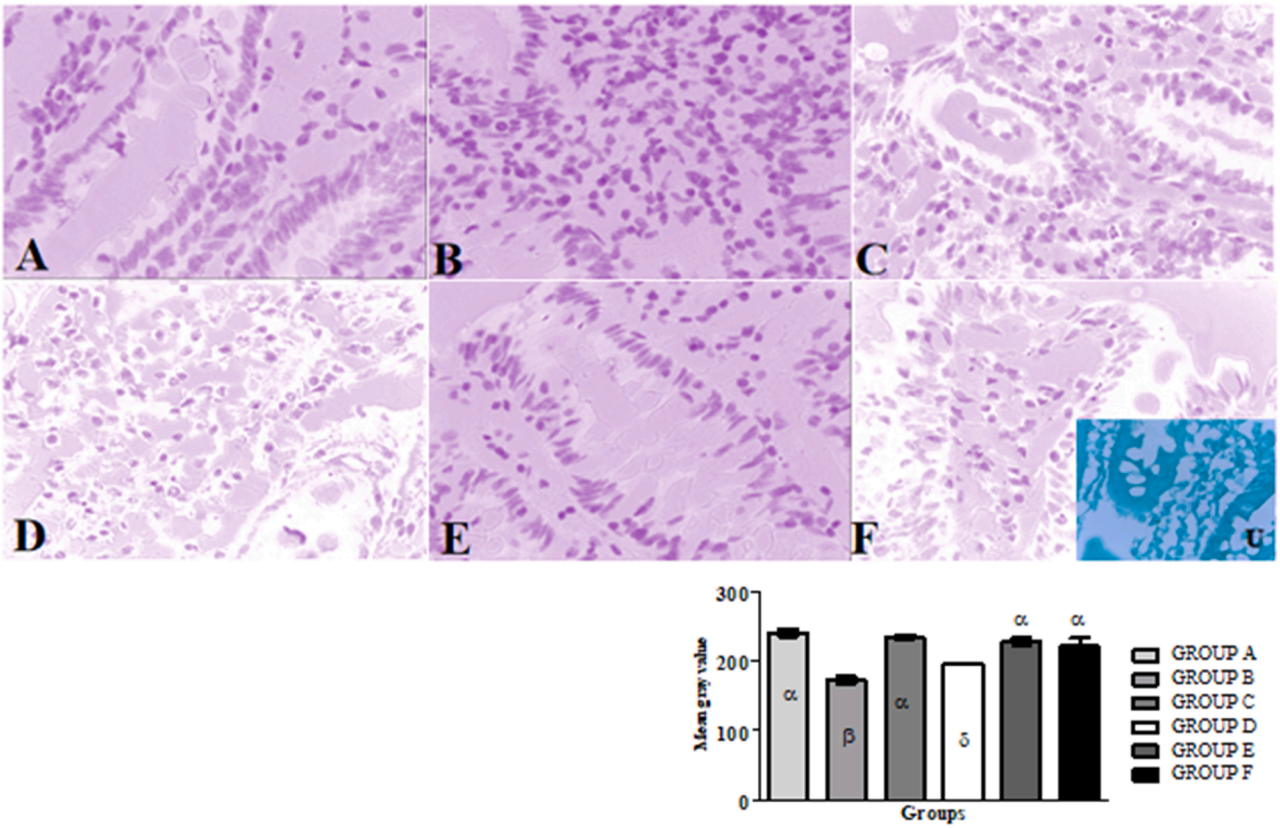

Fig. 5. Representative images of sections of colon subjected to Feulgen reaction for DNA: Observe: colon cells nuclei in groups A-F all show positive reaction to Feulgen while unhydrolyzed control section (inset - U) shows negative reaction to feulgen (Feulgen, 1000X). Bar chart shows staining intensity of nuclear DNA across the groups. *Values are given as Mean ± SEM in each group. Means with different symbols differ significantly while means with the same symbol do not differ significantly.*

Table 3
Nitric oxide level of Aloe gel extract treated acetic acid-induced UC in Rats.

Groups	Mean ± SEM
Group A	4.44 ± 0.12α
Group B	7.04 ± 0.08β
Group C	4.79 ± 0.04α
Group D	6.27 ± 0.12δ
Group E	5.80 ± 0.20γ
Group F	4.77 ± 0.02α

Values are given as Mean ± SEM in each group. Means with different symbols differ significantly while means with the same symbol do not differ significantly.

showed a statistically significant, improvement in rats given Aloe vera for 2 weeks. Finally, the general histology and histochemistry also favored animals treated with Aloe vera gel extract. This research thus provide evidence on the bioactivity and therapeutic effect of aloe gel against UC and suggest that it ameliorate acetic acid-induced UC, through attenuating oxidative stress, and inflammation which it achieved by reducing colon nitric oxide level, in addition to improving histological and Nuclear DNA intensity alterations. It is also worth noting that the remediated potential of aloe gel in acetic acid-induced UC in most cases surpasses that of dexamethasone.

Author's contribution

WOB conducted literature searches, animal experiments, laboratory analyses and analyzed the data. DAO conceptualize and supervised the research project. PA. BAF did literature search and reviewed the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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