Original Article

Thai Journal of Pharmaceutical Sciences

Ocimum gratissimum Linn. (Lamiaceae) protects Wistar rats against inflammation and oxidative stress in trinitrobenzene sulfonic acid-induced colitis

O. O. Abiodun, N. Nwadike, F. N. Ogunleye, A. S. Sosanya

Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria

Corresponding Author:
O. O. Abiodun, Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, PMB 200284, Ibadan, Oyo State, Ibadan. Nigeria.
Tel.: +234/703/096 4774.
E-mail: oyindamolaabiodun1@gmail.com

Received: Sep 15, 2019
Accepted: Feb 26, 2020
Published: Jul 28, 2020

ABSTRACT

Background and Aim: The activity of methanol extract of leaf of *Ocimum gratissimum* a plant with anti-inflammatory, antioxidant, and anti-ulcerogenic activities in a rat model of colitis was evaluated. **Experimental Procedures:** Colitis was induced in 30 of 35 female Wistar rats by intracolonic instillation of 0.25 mL of 40 mg/mL 2, 4, 6-trinitrobenzene sulfonic acid (TNBS). The rats (180–200 g) were distributed into seven groups ($n = 5$): Non-colitic, untreated colitic, and colitic rats treated with prednisolone (2 mg/kg, for 7 days) or pre-treated 2 days before induction of colitis with *O. gratissimum* (50–400 mg/kg) and for additional 7 days post-colitis. Colonic levels of biomarkers of oxidative stress, pro-inflammatory cytokine tumor necrosis factor (TNF-α), myeloperoxidase (MPO) activity, and histology were assessed 24 h post-treatment in euthanized rats. **Results:** *O. gratissimum* at 400 mg/kg produced the highest reduction in colonic damage score, weight/length ratio, malondialdehyde level, TNF-α level, leukocytes infiltration (MPO activity), and prevented reduction of colonic glutathione level compared to prednisolone and other doses of *O. gratissimum* in colitic rats ($P < 0.05$). **Conclusion:** *O. gratissimum* ameliorates TNBS-induced colitis through inhibition of leukocytes infiltration, downregulation of cytokines, and scavenging of free radicals.

Keywords: Colitis, cytokine, myeloperoxidase, malondialdehyde, glutathione

INTRODUCTION

Inflammatory bowel disease (IBD) is a general term used to describe conditions with chronic immune response and inflammation involving the gastrointestinal tract. The two major types of IBD are ulcerative colitis and Crohn's disease (CD).[1,2] The etiology of the disease remains unknown, but it has been hypothesized to be caused by several factors such as genetic predisposition, diet, alterations of the gut microbiome, immunologic abnormalities, and environmental factors.[3]

One of the experimental models that mimic CD to some extent is trinitrobenzene sulfonic acid (TNBS)-induced rat colitis. This model is relevant to CD in terms of pathogenesis, evident by the involvement of NOD2 (a key CD susceptibility gene) in the pathogenesis of TNBS colitis.[4] In TNBS-induced colitis, ethanol is used to disrupt intestinal barrier and enable the interaction of TNBS with colon tissue proteins.[5,6] TNBS acid is a hapten which can couple with proteins with high molecular weight to elicit significant immunologic responses, thereby rendering the proteins immunogenic to the host immune system. A single administration TNBS/EtOH leads to the development of an excessive cell-mediated immune response reflected by acute Th1 inflammation.[7] The phenotype of Th1 inflammation includes a dense colonic tissue infiltration by CD4 T cells and the secretion of various potent pro-inflammatory cytokines.[8] The most characteristic cytokines in that network include tumor necrosis factor-α (TNF-α) and IL-12.[9]

The goals of treatment in IBD include controlling symptoms and maintaining remission, thereby enhancing the quality of life and reducing of cancer risk.[3,10] Treatment of IBD involves the following major classes of drugs; aminosalicylates (5-ASA), e.g., sulfasalazine, mesalazine, etc., corticosteroids (e.g., prednisolone and hydrocortisone), immunomodulators (e.g., methotrexate and azathioprine), and biologics (e.g.,

infliximab and adalimumab, etc.).[11] The benefit of the drugs mentioned above in the treatment of IBD has been proven, but prolong use is related to some serious adverse effects, drug-drug interactions, the risks of infection, and malignancy.[12,13] Thus, there is a need to search for better treatment modalities.

Medicinal plants in the treatment of colitis are put into consideration because plants are sources of biologically active compounds with unequaled chemical diversity that gives opportunity for the development of new drugs.[14,15] *Ocimum gratissimum* Linn. (Lamiaceae) commonly called clove basil, sweet basil, and scent leaf is an herbaceous shrub found in tropical countries, including Nigeria. It is called *Efinrin ajase* in *Yoruba* land in Nigeria.[16] *O. gratissimum* and other species of the genus *Ocimum* such as *O. americanum*, *O. basilicum*, and *O. canum* are reported in Nigeria ethnomedicine for the treatment of bacterial infections, diarrhea, dysentery, and gastrointestinal disorders.[17,18] Biological activities of *O. gratissimum* include antimalarial, antibacterial, antifungal, hypoglycemic, antipyretic, antinociceptive, antidermatophytic, antioxidant, anti-inflammatory, antihelmintic, anticarcinogenic, free radical scavenging, and chemopreventive.[19-21] However, there is limited information on its beneficial effect in TNBS-induced colitis. Thus, this study was embarked on.

MATERIALS AND METHODS

Chemicals and Reagents

Tris-hydroxylamine (Tris-base), potassium chloride (KCl), trichloroacetic acid, thiobarbituric acid (TBA), potassium hydrogentetraoxophosphate (V) (K_2HPO_4), potassium dihydrogentetraoxophosphate (V) (KH_2PO_4), 2,4,6- TNBS acid (TNBS), L-Glutathione (GSH), adrenaline (ADR), 5,5-dithiosbis-2-nitrobenzoate (DTNB), sodium hydrogentrioxocarbonate (IV) decahydrate ($Na_2CO_3.10H_2O$), sodium hydrogen trioxocarbonate (IV) ($NaHCO_3$) O-dianisidine dihydrochloride (ODD), hydrogen peroxide (H_2O_2), and hydrochloric acid (HCl) protease inhibitor cocktail were purchased from Sigma Aldrich (UK). TNF-α assay kit was purchased from BioLegend®, San Diego USA. Ketamine, diazepam, and prednisolone were brought from a reputable local pharmacy store.

Plant Collection and Authentication

Fresh *O. gratissimum* leaf was collected from Adugboku, Igbo-aso Ado-Ekiti. The plant was identified and authenticated at Forestry Herbarium, Forestry Research Institute of Nigeria (FRIN), with an FHI number of 111045.

Plant Extraction

Six hundred grams of fresh plant leaf were air dried and pulverized. The dried plant material was extracted by maceration in 70% methanol for 72 h. The solvent was removed and filtrate concentrated under reduced pressure with a rotary evaporator at 40°C. The percentage yield was 11.83% and extract stored in the refrigerator at 4°C till needed for analysis.

Experimental Animals

Thirty-five adult female Wistar rats weighing 180–200 g were obtained and raised at the animal house, Institute for Advanced Medical Research and Training, University of Ibadan. The rats were kept under room temperature and fed with ACE® standard rat pellets and water *ad libitum*. Experimental procedures and protocols used in this study conform to the "Guide to the care and use of animals in research and teaching" (NIH publications number 85–93 revised in 1985) and the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACCUREC/19/0015).

Induction of Colitis

Female Wistar rats were fasted for 18 h and anesthetized with and ketamine (50 mg/kg)/diazepam (2.5 mg/kg). Thereafter, colitis was induced in 30 of 35 rats by a single intracolonic administration of 0.25 mL of 2, 4, 6- TNBS acid (40 mg/mL) into the distal colon using a catheter inserted 8 cm through the anus according to the method of Morris *et al*.[22] The remaining five rats served as non-colitis group. Following induction of colitis, the rats were held in a head-down position for a few minutes before they were returned to their cages after recovering from anesthesia.

Treatment of Colitic Rats

The methanol extract of *O. gratissimum* was dissolved in distilled water and diluted to a concentration ranging from 50 to 400 mg/mL. The experimental rats were distributed into seven groups ($n = 5$): Non-colitic, untreated colitic, and colitic rats treated with prednisolone (2 mg/kg, for 7 days) or pre-treated 2 days before induction of colitis with *O. gratissimum* (50, 100, 200, and 400 mg/kg) and for additional 7 days post-colitis. Plant extract and prednisolone were given orally.

Disease Activity Index (DAI)

DAI was used to quantify the evolution of the TNBS-induced colitis in experimental mice using a modification of a previously reported method.[23] The body weight, occurrence of diarrhea/rectal bleeding, and food intake were recorded for each mouse throughout the period of the study starting day 1 post-colitis induction. These parameters were assigned a score and utilized to calculate the average daily DAI [Table 1].

Evaluation of the Activity of Methanol Extract of *O. gratissimum* in Rat Colitis

Rats were euthanized 7 days after the induction of colitis. Cold normal saline was used to flush the content of the rat's distal colon. Thereafter, an incision was made on the colon along the mesenteric border, the surrounding tissues or fat were removed. Colon was weighed and the length measured. Disease severity such as hyperemia, swelling, ulcer, inflammation, and necrosis was assessed using a standard scoring system on a scale of 0–10.[24] Subsequently, samples of the colon for histological examinations, biochemical assays, and TNF-α estimation were cut from the distal colon.

Histopathological Examination

A section of the colon was cut and fixed in 10% formalin individually, dehydrated, and embedded in paraffin. The processed colon tissue was sectioned in slices and stained with hematoxylin and eosin.[25]

Table 1: Disease activity index scoring system

Score	Percentage decrease in weight (%)	Decrease in food intake (g)	Stool consistency/rectal bleeding
0	0	0–5	Solid (normal) stool
1	1–5	6–10	Semi-solid stool
2	6–10	11–15	Slightly loose stool
3	11–15	16–20	Very loose stool
4	>15	>20	Diarrhea with blood and/or mucus

Biochemical Assays

The remaining colon was minced thoroughly on an ice pack. Aliquot of minced colon was put into three Eppendorf tubes and stored at −80°C. Minced colon in tube 1 was homogenized in 10 mM hexadecyltrimethylammonium bromide buffer (50 mg/mL). Reduced GSH and lipid peroxidation levels were estimated from the supernatant from the homogenized colon. Reduced GSH level was estimated according to a previously reported method[26] and the result expressed as nanomole per gram of wet tissue. Lipid peroxidation was assayed by measuring the TBA acid-reactive products present in the sample using a previously reported method.[27] The result was expressed as micromole of malondialdehyde (MDA) per gram tissue. The remaining supernatant and tissue were subjected to two cycles of freeze-thaw process and used for estimating myeloperoxidase (MPO) activity. The method described by Krawisz *et al.*[28] was used to measure MPO activity and the result was expressed as MPO units per gram of wet tissue. One unit of MPO activity was defined as that that will degrade 1 mmol hydrogen peroxide/min at 25°C.

Determination of Colon Tumor Necrosis Factor-alpha (TNF-α)

A known portion of the minced colon in tube 2 (50 mg) was homogenized in 1 mL lysis buffer containing protease inhibitor. Homogenates were centrifuged at 12,000 rpm for 10 min at 4°C. An enzyme-linked immunosorbent assay kit was used to estimate colonic TNF-α following the manufacturer's instruction (BioLegend, San Diego, USA).

Statistical Analysis

All results were expressed as mean ± standard error of mean. Data were analyzed by one way ANOVA followed by Dunnett's test to compare treatment groups with the control group, using GraphPad® Prism 5 software. Statistical significance was taken at $P < 0.05$.

RESULTS

Effects of Leaf Methanol Extract of *O. gratissimum* on DAI Score in Colitis Rats

TNBS/ethanol administration to rats resulted in bloody diarrheal, reduced food intake, and significant weight loss [Figure 1]. The DAI scored was high 24 h after induction of colitis. However, treatment with leaf methanol extract of *O. gratissimum* significantly decreased the DAI score compared to untreated colitic rats [$P < 0.05$, Figure 1].

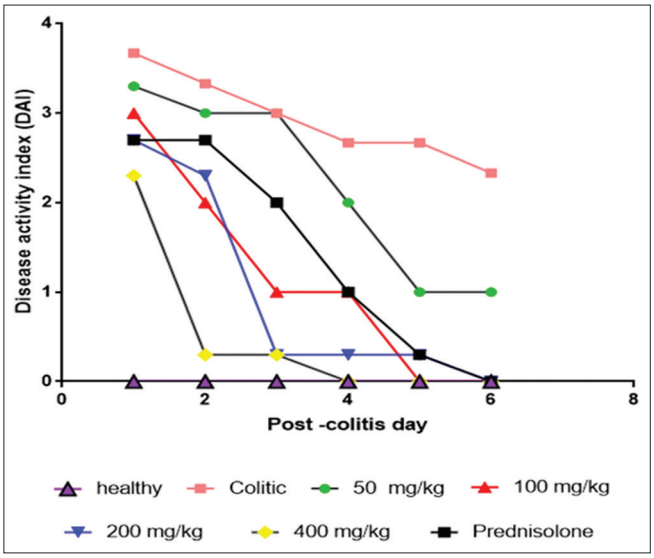


Figure 1: Effect s of methanol extract of *Ocimum gratissimum* on disease activity index in trinitrobenzene sulfonic acid-induced colitic rats.

The Effect of Methanol Extract of Leaf of *O. gratissimum* on Macroscopic Colonic Damage Score and Weight/Ratio

Hyperemia, edema, inflammation, ulceration, or necrosis were observed in the colon of some experimental rats. These signs were scored on a previously reported scale 0–10. Treatment with *O. gratissimum* (100–400 mg/kg) and prednisolone (2 mg/kg) resulted in significantly lower colonic damage score ranging from of 3.33 ± 0.33 to 4.88 ± 0.38 compared to untreated colitic group 6.33 ± 0.4 [$P \leq 0.03$; Table 2]. In addition, the colonic weight/length ratio of the untreated colitic group (266.33 ± 8.80 mg/cm) was significantly higher than that of the non-colitic group (122.0 ± 9.15 mg/cm; $P < 0.0001$). The weight/length ratios of the colon of the rats treated with 2 mg/kg prednisolone, 200 and 400 mg/kg of *O. gratissimum* (176.99 ± 6.22, 178.17 ± 2.11 and 172.0 ± 15.0 mg/cm, respectively), were significantly lower than the untreated colitic group $P \leq 0.0006$, Table 2).

The Effect of Methanol Extract of Leaf of *O. gratissimum* on Colonic Myeloperoxidase (MPO) Activity

Furthermore, increased MPO activity was observed in untreated colitic rats (12.61 ± 1.29 mU/mg tissue) than non-colitic group (1.14 ± 0.13 mU/mg tissue). It was observed that treatment with 400 mg/kg *O. gratissimum* (2.79 ± 0.29 mU/mg tissue) produced the highest reduction in colonic MPO activity than prednisolone

Table 2: The effects of methanol extract of *Ocimum gratissimum* on colonic damage and colonic weight/length ratio in TNBS-induced colitic rats

Treatment groups	Colonic damage score	Colonic weight/length ratio (mg/cm)
Non-colitic	0.0±0.0#	122.0±9.15*
Colitic	6.33±0.4	266.33±8.80
Colitic + prednisolone 2 mg/kg	4.50±0.5#	176.99±6.22*
Colitic + Og 50 mg/kg	5.63±0.44	214.15±6.38
Colitic + Og 100 mg/kg	4.88±0.38#	200.11±3.62
Colitic + Og 200 mg/kg	4.60±0.24#	178.17±2.11*
Colitic + Og 400 mg/kg	3.33±0.33#	172.0±15.0*

n=5, Result presented as mean±SEM (standard error of mean), Og – *Ocimum gratissimum* #colitic group versus other groups $P \leq 0.03$, *colitic group versus other groups $P \leq 0.0012$

(4.80 ± 0.74 mU/mg tissue) and the other treatment groups (5.88 ± 0.84–6.83 ± 1.61 mU/mg tissue; Figure 2).

The Effect of Methanol Extract of Leaf of *O. gratissimum* on Colonic Levels of Biomarkers of Oxidative Stress

Significant depletion of colonic GSH occurred in untreated colitic rats (4.53 ± 0.7 nmol/g tissue) compared to rats without colitis (81.1 ± 1.4 nmol/g tissue, $P < 0.0001$). Treatment with *O. gratissimum* (200 and 400 mg/kg) prevented depletion of colonic GSH content in colitic rats (70.67 ± 6.93 and 109.88 ± 6.35 nmol/g tissue) better than prednisolone (41.53 ± 0.70 nmol/g tissue) and the other treatment groups (10.73 ± 1.95–34.79 ± 6.13 nmol/g tissue, $P < 0.005$; Figure 3). In addition, MDA level increased significantly in untreated colitic group (14.42 ± 0.68 µmol/g tissue) compared to non-colitic treatment group (1.86 ± 0.52 µmol/g tissue, $P < 0.0001$). *O. gratissimum* at 400 mg/kg produced the highest reduction in MDA level (3.58 ± 0.48 µmol/g tissue) than prednisolone and the other treatment groups (8.72 ± 0.64–11.93 ± 1.41 µmol/g tissue, Figure 4).

The Effect of Methanol Extract of Leaf of *O. gratissimum* on Colonic TNF-α Level

Pro-inflammatory cytokine, TNF-α activity was significantly elevated in the colon of the colitic untreated rats (179 ± 11.98 pg/mg tissue) than in the non-colitic group (44.22 ± 1.70 pg/mg tissue, $P = 0.0004$). There was also a significant reduction in colonic TNF-α content in the groups that received *O. gratissimum* extract and prednisolone [Figure 5]. However, 400 mg/kg *O. gratissimum* (40.48 ± 0.88 pg/mg tissue) produced the most remarkable reduction that is comparable to the level of the colonic TNF-α found in the non-colitic group ($P = 0.09$).

The Effect of Methanol Extract of Leaf of *O. gratissimum* on the Histology of the Colon of the Colitic Rats

Microscopic examination of the mucosa of the colon revealed normal architecture with preserved epithelial layers, well-organized crypts with goblets, and lamina propria. No inflammatory infiltrate or necrosis was seen in the non-colitic group [Figure 6a]. On the other hand, the colon of untreated colitic rats showed severe inflammation and

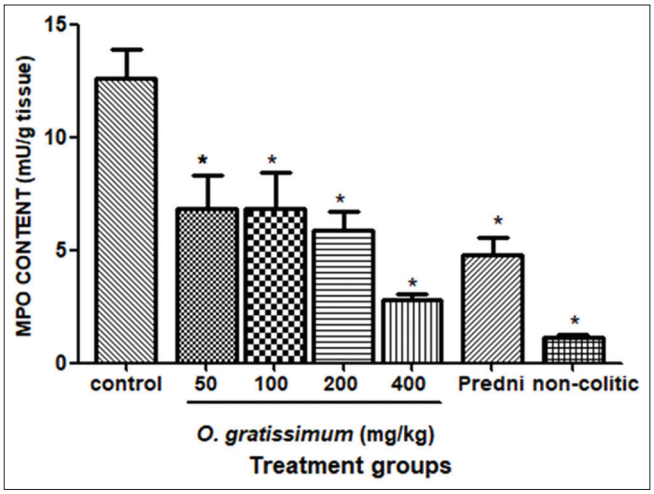


Figure 2: Effects of methanol extract of *Ocimum gratissimum* on colonic myeloperoxidase (MPO)activity level in trinitrobenzene sulfonic acid-induced colitic rats. Each column represents mean ± SEM ($n = 5$), predni = prednisolone, *significance reduction of MPO in colitic rats pre-treated with *O. gratissimum* or pred compared to control ($P < 0.05$)

widespread mucosal damage characterized by the infiltration of mononuclear leukocytes (lymphocytes and macrophages). The structure of the crypts was distorted and mucosa eroded [Figure 6b]. Colitic rats treated with prednisolone or 400 mg/kg *O. gratissimum* showed improvement in their colonic histology, the architecture of the mucosal was preserved, goblet cells are well preserved, and there was a few cellular infiltration in the mucosa with near intact lamina propria [Figure 6].

DISCUSSION

This study confirmed that TNBS dissolved in 50% ethanol administered into the rat colon resulted in ulceration and inflammation of the rat colon. This rat model of TNBS-induced colitis results in a dysfunction T cell-mediated immunity and the generation of oxygen radicals.[29] The disease involves leukocytes infiltration and eventual generation of pro-inflammatory cytokines.[7,9] Oral administration of *O. gratissimum* curtailed colonic damages, which is an index of disease severity, with 400 mg/kg producing the maximum beneficial effects. The reduction of the colonic weight/length ratio, a marker of edema, a

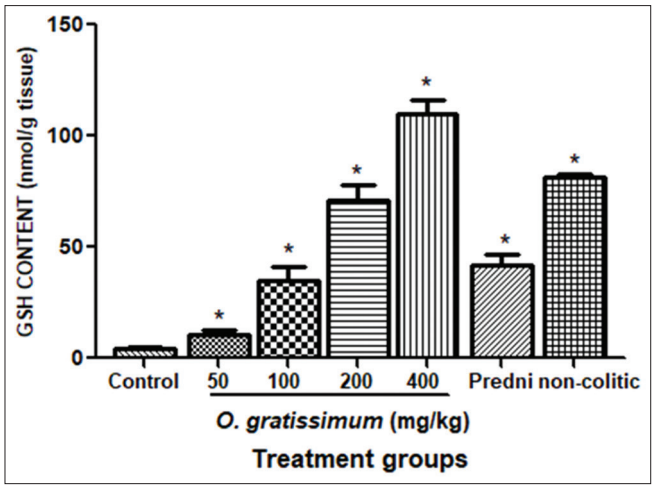


Figure 3: Effects of methanol extract of *Ocimum gratissimum* on colonic glutathione (GSH) level in trinitrobenzene sulfonic acid-induced colitic rats. Each column represents the mean ± SEM (n = 5), predni = prednisolone, *significance increase in GSH content in colitic rats pre-treated with *O. gratissimum* or pred compared to control ($P < 0.05$)

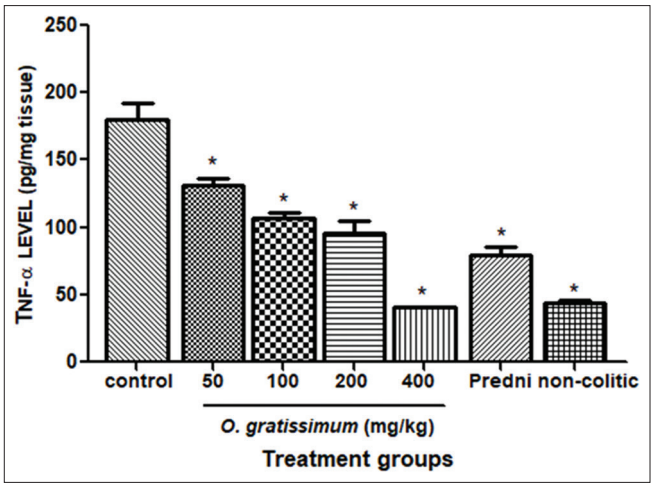


Figure 5: Effects of methanol extract of *Ocimum gratissimum* on colonic tumor necrosis factor-α (TNF-α) level in trinitrobenzene sulfonic acid-induced colitic rats. Each column represents the mean ± SEM (n = 5), *significance reduction of TNF-α level in colitic rats pre-treated with *O. gratissimum* or pred compared to control ($P < 0.05$)

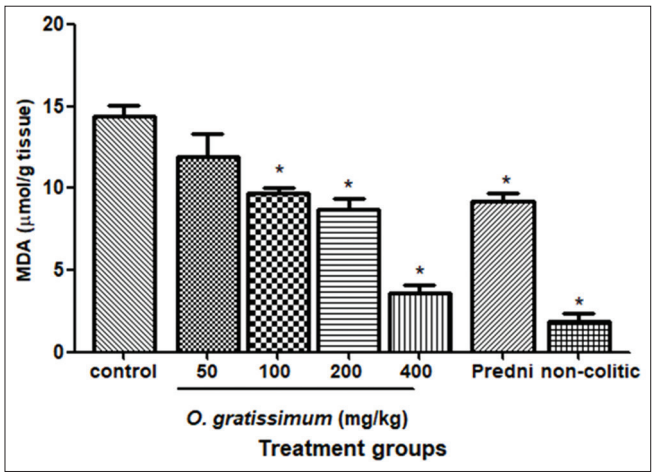


Figure 4: Effects of methanol extract of *Ocimum gratissimum* on colonic malondialdehyde (MDA) level in trinitrobenzene sulfonic acid-induced colitic rats. Each column represents the mean ± SEM (n = 5), predni = prednisolone, *significance reduction of MDA in colitic rats pre-treated with *O. gratissimum* (100–400 mg/kg) or pred compared to control ($P < 0.05$)

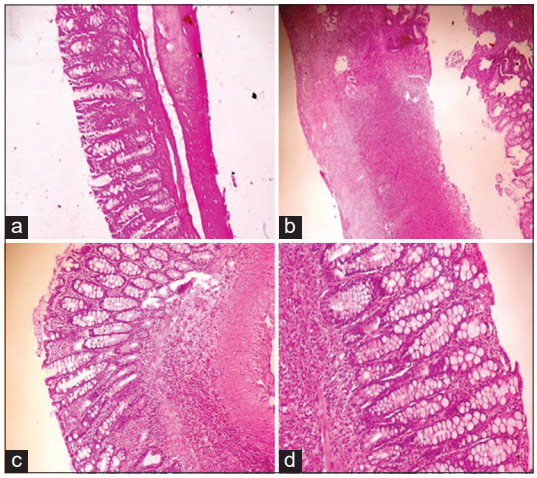
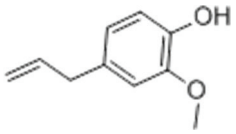
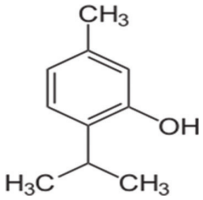
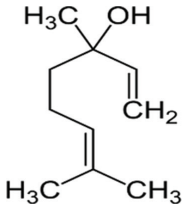
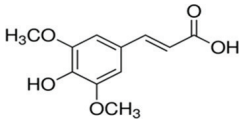
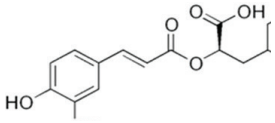
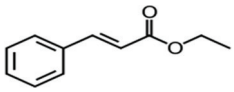
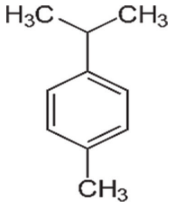
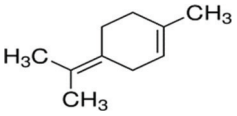
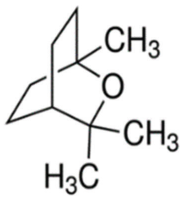
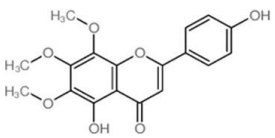


Figure 6: Histologic section of colon section stained with hematoxylin and eosin, magnification (×100), showing the effect of *Ocimum gratissimum* in trinitrobenzene sulfonic acid-induced colitis: (a) Non-colitic rats, (b) untreated colitic rats, (c) colitic rat treated with prednisolone, and (d) colitic rat treated with 400 mg/kg *O. gratissimum*

symptom of inflammation was observed with *O. gratissimum* treatment.

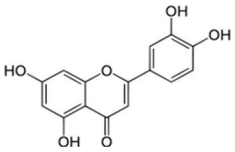
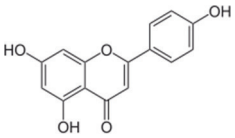
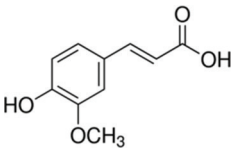
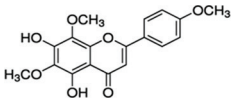
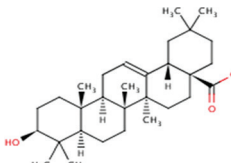
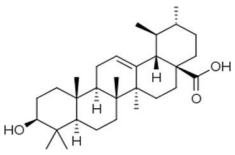
Reactive oxygen species plays a major role in the initiation and progression of IBD by attacking the cellular macromolecules.[30] Consequently causing the disruption of epithelial cell integrity and hindering mucosal recovery, particularly when there is an impairment of the endogenous defense systems.[31,32] *O. gratissimum* extract-treated colitic rats showed a significant reduction in the levels of colonic MDA, which signified a reduction in lipid peroxidation and therefore an increase in the stability of the cellular membrane. An increase in lipid peroxidation in the colonic tissues leads to a process that generates more reactive metabolites which deplete cellular antioxidants and further potentiate inflammation. Interestingly, in this study, treatment with *O. gratissimum* resulted in the prevention of the depletion of GSH an antioxidant. Prevention of the depletion of cellular antioxidants will protect the already inflamed mucosa and accelerate tissue recovery.[33] MPO, an index of infiltration of neutrophils increased in TNBS-induced colitis in this study, which is similar to previous reports.[34] However, MPO activity was inhibited by *O. gratissimum*. In addition, colonic levels of pro-inflammatory cytokine TNF-α were significantly increased in colitic untreated rats compared to non-colitic rats. *O. gratissimum* decreased the production of colonic TNF-α in colitic rats. TNF-α has been shown to aid the infiltration of inflammatory cells by encouraging the binding of neutrophils and lymphocytes to endothelial cells.[35] Thus, methanol extract of *O. gratissimum* displayed anti-inflammatory activity

Table 3: Major phytochemical constituents found in *Ocimum gratissimum* leaf

S. No.	Name of compounds	Structure of compounds	Molecular weight (g/mol)	Pharmacological activity	References
1.	Eugenol		164.2	Anti-inflammatory, antipyretic, antioxidant, and analgesic properties	[36,37]
2.	Thymol		150.22	Anti-inflammatory, anti-obesity, antibacterial	[38-40]
3.	Linalool		154.25	Anti-inflammatory, antinociceptive antihyperalgesic, and antioxidant	[41,42]
4.	Sinapic acid		224.21	Anti-inflammatory, antioxidant, weak antimicrobial activities	[43,44]
5.	Rosmarinic acid		360.3	Anti-inflammatory, immunomodulatory, anti-oxidant, neuroprotection	[45,46]
6.	Ethyl cinnamate		176.21	Antioxidant, antifungal	[47,48]
7.	p-cymene		134.22	Antioxidant, antinociceptive and anti-inflammatory	[49,50]
8.	Terpinolene		136.23	Wound healing, anti-inflammatory, and antioxidant	[51,52]
9.	1,8-cineole		154.253	Antinociceptive and anti-inflammatory ulcer healing agent, antioxidant and cytoprotective	[53,54]
10.	Xanthomicrol		344.3	Anti-spasmodic, anti-platelet and anti-cancer effect	[55]

(*Contd...*)

Table 3: (*Continued*)

S. No.	Name of compounds	Structure of compounds	Molecular weight (g/mol)	Pharmacological activity	References
11.	Luteolin		286.24	Anti-inflammatory, antioxidant, antimicrobial, and anticancer activities	[56,57]
12.	Apigenin		270.24	Anti-inflammatory, anti-toxicant, anti-cancer	[58]
13.	Trans-ferulic acid		194.18	Antioxidant, anti-inflammatory, antimicrobial, anti-allergic, hepatoprotective, anticarcinogenic, antithrombotic, antiviral, and vasodilatory actions	[59,60]
14.	Nevadensin		344.32	Hypotensive, antimicrobial, anti-inflammation, and anti-cancer activities	[61,62]
15.	Oleanolic acid		456.71	Hepatoprotective, antioxidant, anticancer, and anti-inflammatory	[63-65]
16.	Ursolic acid		456.7	Anti-inflammatory, antitumor antioxidant, anti-HIV, and anti-*Mycobacterium tuberculosis* effects	[66,67]

by preventing upregulation of TNF-α, thereby reducing the activity of MPO.

Previous reports of biologically active compounds in the leaf of *O. gratissimum* abound. Some of the major compounds and pharmacological activities previously reported are presented in Table 3. The leaf is rich in essential oil such as eugenol, thymol, and linalool with anti-inflammatory, antipyretic, antinociceptive, antihyperalgesia, antioxidant, and other pharmacological activities.[36-42] In addition, sinapic acid, rosmarinic acid, ethyl cinnamate, p-cymene, terpinolene, and 1, 8-cineone are components of the leaf of *O. gratissimum* with antioxidant, antinociceptive, cytoprotective, ulcer healing, or wound healing.[43-54] Other compounds such as xanthomicrol, luteolin, apigenin, and trans-ferulic acid are associated with anticancer, anti-toxicant, antioxidant, anti-inflammatory, antimicrobial, anti-allergic, or hepatoprotective effects.[55-60] Nevadensin, oleanolic acid, and ursolic acid reported in literature are also bioactive constituents of *O. gratissimum* with various activities such as anti-inflammatory, anti-tuberculosis, antitumor, antioxidant, and anti-inflammatory activities.[61-67] The presence of these pharmacologically active compounds in *O. gratissimum* supports its medicinal use in the management of inflammatory and oxidative disorders in IBD.

CONCULSION

The methanol extract of *O. gratissimum* resolved intestinal inflammation and oxidative stress in TNBS-induced rat's colitis. The mechanism of activity appears to be through inhibition of neutrophils, downregulation of cytokines, and scavenging of free radicals.

CONFLICTS OF INTEREST STATEMENT

All authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The technical assistance of Mrs. Sakirat Braimah of *in vivo* animal section of Malaria Research Laboratory, University of Ibadan during the course of the study, is acknowledged.

REFERENCES

1. Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, *et al.* Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology 2012;142:46-54.
2. Tian T, Wang Z, Zhang J. Pathomechanism of oxidative stress in inflammatory bowel disease and potential antioxidant therapies.

Oxid Med Cell Longev 2017;2017:4535194.
3. Singh UP, Singh NP, Busbee B, Guan H, Singh B, Price RL, *et al.* Alternative medicines as emerging therapies for inflammatory bowel diseases. Int Rev Immunol 2012;31:66-84.
4. Watanabe T, Asano N, Murray PJ, Ozato K, Tailor P, Fuss IJ, *et al*. Muramyl dipeptide activation of nucleotide-binding oligomerization domain 2 protects mice from experimental colitis. J Clin Investig 2018;118:545-59.
5. Neurath MF, Fuss I, Kelsall BL, Stüber E, Strober W. Antibodies to interleukin 12 abrogate established experimental colitis in mice. J Exp Med 1995;182:1281-90.
6. Ikeda M, Takeshima F, Isomoto H, Shikuwa S, Mizuta Y, Ozono Y, *et al*. Simvastatin attenuates trinitrobenzene sulfonic acid-induced colitis, but not oxazalone-induced colitis. Dig Dis Sci 2008;53:1869-75.
7. Elson CO, Sartor RB, Tennyson GS, Riddell RH. Experimental models of inflammatory bowel disease. Gastroenterology 1995;109:1344-67.
8. da Silva MS, Sanchez-Fidalgo S, Talero E, Cardeno A, da Silva MA, Villegas W, *et al*. Anti-inflammatory intestinal activity of *Abarema cochliocarpos* (Gomes) Barneby and Grimes in TNBS colitis model. J Ethnopharmacol 2010;128:467-75.
9. Takagi T, Naito Y, Mizushima K, Akagiri S, Suzuki T, Hirata I, *et al*. Inhalation of carbon monoxide ameliorates TNBS-induced colitis in mice through the inhibition of TNF-alpha expression. Dig Dis Sci 2010;55:2797-804.
10. Kornbluth A, Sachar DB. Ulcerative colitis practice guidelines in adults: American college of gastroenterology, practice parameters committee. Am J Gastroenterol 2010;105:501-23.
11. Hanauer SB. Review article: Evolving concepts in treatment and disease modification in ulcerative colitis. Aliment Pharmacol Ther 2008;27:15-21.
12. de Mattos BR, Garcia MP, Nogueira JB, Paiatto LN, Albuquerque CG, Souza CL, *et al*. Inflammatory bowel disease: An overview of immune mechanisms and biological treatments. Mediat Inflamm 2015;2015:493012.
13. Lichtenstein GR, Rutgeerts P, Sandborn WJ, Sands BE, Diamond RH, Blank M, *et al.* A pooled analysis of infections, malignancy, and mortality in infliximab and immunomodulator-treated adult patients with inflammatory bowel disease. Am J Gastroenterol 2012;107:1051-87.
14. Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: How to develop a stronger *in vitro* proof of concept. J Ethnopharmacol 2006;106:290-302.
15. Ng SC, Lam YT, Tsoi KK, Chan FK, Sung JJ, Wu JC. Systematic review: The efficacy of herbal therapy in inflammatory bowel disease. Aliment Pharmacol Ther 2013;38:854-63.
16. Ladipo MK, Doherty VF, Kanife UC. Phytochemical screening and antibacterial investigation of the extract of *Ocimum gratissimum* (scent leaf) on selected *Enterobacteriaceae*. PAT 2010;6:75-84.
17. Sofowora A. Medicinal Plants and Traditional Medicinal Medicine in African. Ibadan: Spectrum Books Ltd.; 1993. p. 191-289.
18. Idika N, Niemogha MT. The diversity of uses of Medicinal plants in Nigeria. In: Odugbemi T, editor. A Textbook of Medicinal Plants from Nigeria. Lagos: University of Lagos Press; 2008.
19. Janssen AM, Scheffer JJ, Ntezurubanza L, Svendsen AB. Antimicrobial activities of some ocimum species grown in Rwanda. J Ethnopharmacol 1989;26:57-63.
20. Prabhu KS, Lobo R, Shirwaikar AA, A. Shirwaikar A. *Ocimum gratissimum*: A review of its chemical, pharmacological and ethnomedicinal properties. Open Complement Med J 2009;1:1-15.
21. Ajayi AM, Umukoro S, Ben-Azu B, Adzu B, Ademowo OG. Toxicity and protective effect of phenolic-enriched ethyl acetate fraction of *Ocimum gratissimum* (Linn.) leaf against acute inflammation and oxidative stress in rats. Drug Dev Res 2017;78:135-45.
22. Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterol 1989;96:795-803.
23. Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest 1993;69:238-49.
24. Camuesco D, Peran L, Comalada M, Nieto A, Di Stasi LC, Rodriguez-Cabezas ME, *et al.* Preventative effects of lactulose in the trinitrobenzene sulphonic acid model of rat colitis. Inflamm Bowel Dis 2005;11:265-71.
25. Arribas B, Suarez-Pereira E, Mellet CO, Fernández JM, Buttersack C, Rodríguez-Cabezas ME, *et al*. Di-D-fructose dianhydride-enriched caramels: Effect on colon microbiota, inflammation, and tissue damage in trinitrobenzene sulfonic acid-induced colitic rats. J Agric Food Chem 2010;58:6476-84.
26. Anderson ME. Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol 1985;113:548-55.
27. Varshney R, Kale RK. Effect of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. Int J Radiat Biol 1990;58:733-43.
28. Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity assessment of inflammation in rat and hamster models. Gastroenterol 1984;87:1344-50.
29. Wendel GH, Maria AO, Domínguez NS, Mohamed F, Scardapane L, Guzman JA, *et al.* Intrarectal dehydroleucodine suppresses trinitrobenzene sulfonic acid induced colitis in rats, a model for inflammatory bowel disease. Pharm Biol 2007;45:316-23.
30. Kruidenier L, Verspaget V. Review article: Oxidative stress as a pathogenic factor in inflammatory bowel disease radicals or ridiculous? Aliment Pharmacol Ther 2002;16:1997-2015.
31. Buffinton GD, Doe WF. Altered ascorbic acid status in the mucosa from inflammatory bowel disease patients. Free Radic Res 1995;22:131-43.
32. Joshi SV, Vyas BA, Shah PD, Shah DR, Shah SA, Gandhi TR. Protective effect of aqueous extract of *Oroxy lumindicum* Linn. (root bark) against DNBS-induced colitis in rats. Indian J Pharmacol 2011;43:656-61.
33. Abiodun OO, Rodríguez-Nogales A, Algieri F, Gomez-Caravaca AM, Segura-Carretero A, Utrilla MP, *et al.* Antiinflammatory and immunomodulatory activity of an ethanolic extract from the stem bark of *Terminalia catappa* L. (*Combretaceae*): *In vitro* and *in vivo* evidences. J Ethnopharmacol 2016;192:309-19.
34. Randhawa PK, Singh K, Singh N, Jaggi AS. A review on chemical induced inflammatory bowel disease models in rodents. Korean J Physiol Pharmacol 2014;18:279-88.
35. Algieri F, Rodríguez-Nogales A, Garrido-Mesa N, Utrilla MP, Montilla A, Cardelle-Cobas A, *et al.* Intestinal anti-inflammatory effects of oligosaccharides derived from lactulose in the trinitrobenzene sulfonic acid model of rat colitis. J Agric Food Chem 2014;62:4285-97.
36. Dal Bo W, Luiz AP, Martins DF, Mazzardo-Martins L, Santos AR. Eugenol reduces acute pain in mice by modulating the glutamatergic and tumor necrosis factor alpha (TNF-alpha) pathways. Fundam Clin Pharmacol 2013;27:517-25.
37. Fonseca DV, Salgado PR, Humberto CA, Golzio AM, Filho MC, Melo CG, *et al*. Ortho-eugenol exhibits anti-nociceptive and anti-inflammatory activities. Int Immunopharmacol 2016;38:402-8.
38. Zhou E, Fu Y, Wei Z, Yu Y, Zhang X, Yang Z. Thymol attenuates allergic airway inflammation in ovalbumin (OVA)-induced mouse asthma. Fitoterapia 2014;96:131-7.
39. Haque MR, Ansari SH, Najmi AK, Ahmad MA. Monoterpene phenolic compound thymol prevents high fat diet induced obesity in murine model. Toxicol Mech Methods 2014;24:116-23.
40. Chauhan AK, Kang SC. Thymol disrupts the membrane integrity of *Salmonella* ser. Typhimurium *in vitro* and recovers infected macrophages from oxidative stress in an *ex vivo* model. Res

Microbiol 2014;165:559-65.
41. Peana AT, D'Aquila PS, Panin F, Serra G, Pippia P, Moretti MD. Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. Phytomedicine 2002;9:721-6.
42. Peana AT, De Montis MG, Sechi S, Sircana G, D'Aquila PS, Pippia P. Effects of (-)-linalool in the acute hyperalgesia induced by carrageenan, L-glutamate and prostaglandin E2. Eur J Pharmacol 2004;497:279-84.
43. Yun KJ, Koh DJ, Kim SH, Park SJ, Ryu JH, Kim DG, *et al*. Antiinflammatory effects of sinapic acid through the suppression of inducible nitric oxide synthase, cyclooxygase-2, and proinflammatory cytokines expressions via nuclear factor-κB inactivation. J Agric Food Chem 2008;56:10265-72.
44. Hameed H, Aydin S, Başaran N. Sinapic acid: Is it safe for humans? FABAD J Pharm Sci 2016;41:39-49.
45. Oh HA, Park CS, Ahn HJ, Park YS, Kim HM. Effect of *Perilla frutescens* var. *acuta* Kudo and rosmarinic acid on allergic inflammatory reactions. Exp Biol Med 2011;236:99-106.
46. Fernando PM, Piao MJ, Kang KA, Ryu YS, Hewage SR, Chae SW, *et al*. Rosmarinic acid attenuates cell damage against UVB radiation-induced oxidative stress via enhancing antioxidant effects in human HaCaT cells. Biomol Ther 2016;24:75-84.
47. Zhou K, Chen D, Li B, Zhang B, Miao F, Zhou L. Bioactivity and structure-activity relationship of cinnamic acid esters and their derivatives as potential antifungal agents for plant protection. PLoS One 2017;12:0176189.
48. Rafiq L, Razia S, Koul KK. Role of cinnamate and cinnamate derivatives in pharmacology. Global J Pharmacol 2013;8:328-35.
49. de Oliveira TM, de Carvalho RB, da Costa IH, de Oliveira GA, de Souza AA, de Lima SG, *et al*. Evaluation of p-cymene, a natural antioxidant. Pharm Biol 2015;53:423-8.
50. Quintans Jde S, Menezes PP, Santos MR, Bonjardim LR, Almeida JR, Gelain DP, *et al*. Improvement of p-cymene antinociceptive and anti-inflammatory effects by inclusion in β-cyclodextrin. Phytomedicine 2013;20:436-40.
51. de Christo Scherer MM, Marques FM, Figueira MM, Peisino MC, Schmitt EF, Kondratyuk TP, *et al*. Wound healing activity of terpinolene and α-phellandrene by attenuating inflammation and oxidative stress *in vitro*. J Tissue Viability 2019;28:94-9.
52. Macedo EM, Santos WC, Sousa BP, Lopes EM, Piauilino CA, Cunha FV, *et al*. Association of terpinolene and diclofenac presents antinociceptive and anti-inflammatory synergistic effects in a model of chronic inflammation. Braz J Med Biol Res 2016;49:e5103.
53. Rocha Caldas GF, Oliveira AR, Araújo AV, Lafayette SS, Albuquerque GS, Silva-Neto J, *et al*. Gastroprotective mechanisms of the monoterpene 1,8-Cineole (Eucalyptol). PLoS One 2015;10:e0134558.
54. Santos FA, Rao VS. Antiinflammatory and antinociceptive effects of 1,8 cineole a terpenoid oxide present in many plant essential oils. Phytother Res 2000;14:240-4.
55. Fattahi M, Cusido RM, Khojasteh A, Bonfill M, Palazon J. Xanthomicrol: A comprehensive review of its chemistry, distribution, biosynthesis and pharmacological activity. Mini Rev Med Chem 2014;14:725-33.
56. Aziz N, Kim MY, Cho JY. Anti-inflammatory effects of luteolin: A review of *in vitro*, *in vivo*, and *in silico* studies. J Ethnopharmacol 2018;225:342-58.
57. López-Lázaro M. Distribution and biological activities of the flavonoid luteolin. Mini Rev Med Chem 2009;9:31-59.
58. Ali F, Naz FR, Jyoti S, Siddique HY. Health functionality of apigenin: A review. Int J Food Properties 2017;20:1197-238.
59. Batista R. Uses and potential applications of ferulic acid. In: Warren B, editor. Ferulic Acid: Antioxidant Properties, Uses and Potential Health Benefits. 1st ed. New York: Nova Science Publishers, Inc.; 2014. p. 39-70.
60. Kumar N, Pruthi V. Potential applications of ferulic acid from natural sources. Biotechnol Rep (Amst) 2014;4:86-93.
61. Brahmachari G. Nevadensin: Isolation, chemistry and bioactivity. Int J Green Pharm 2010;4:213-9.
62. Song JY, He XZ, Chen XF, Hu JY, Luo GY, Mo YZ. Hypotensive effect of nevadensin. Acta Pharmacol Sin 1985;6:99-102.
63. Liu J. Pharmacology of oleanolic acid and ursolic acid. J Ethnopharmacol 1995;49:57-68.
64. Wang X, Ye X, Liu R, Chen HL, Bai H, Liang X, *et al*. Antioxidant activities of oleanolic acid *in vitro*: Possible role of Nrf2 and MAP kinases. Chem Biol Interact 2010;184:328-37.
65. Pollier J, Goossens A. Oleanolic acid. Phytochemistry 2012;77:10-5.
66. Baricevic D, Sosa S, Loggia RD, Tubaro A, Simonovska B, Krasna A, *et al*. Topical anti-inflammatory activity of *Salvia officinalis* L. leaves: The relevance of ursolic acid. J Ethnopharmacol 2001;75:125-32.
67. Mlala S, Oyedeji AO, Gondwe M, Oyedeji OO. Ursolic acid and its derivatives as bioactive agents. Molecules 2019;24:E2751.