

Ocimum gratissimum diet inclusion abrogates N-methyl-N-nitrosourea-induced colorectal toxicity in male Wistar rats via attenuating oxidative stress and colonic mucosa injuries

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ABSTRACT

Background: Colorectal toxicity disrupts intestinal homeostasis, undermining treatment efficacy and diminishing patient quality of life. The preventive effects of dietary inclusion of graded levels of *Ocimum gratissimum* leaf on N-methyl-N-nitrosourea (MNU)-induced colorectal toxicity were evaluated in male Wistar rats.

Methods: Thirty male rats (70–90 g) were acclimatized for two weeks and randomly assigned to six groups (n = 5). All groups, except the Normal control, and the 10% diet control received intrarectal MNU (9mg/kgbw) for 10 weeks together with diets containing 0%, 2.5%, 5%, or 10% *O. gratissimum* leaf. At the end of the experiment, rats were sacrificed and blood and organ tissues were collected for carcinoembryonic antigen (CEA) determination, histopathological examination, and biochemical assays.

Results: Carcinoembryonic antigen (CEA) levels were significantly higher in the MNU control group than in normal controls ($P < 0.05$), but decreased progressively with graded dietary *O. gratissimum* supplementation. Dietary *O. gratissimum* significantly suppressed the MNU-induced rise in colonic malondialdehyde ($P < 0.05$) and concomitantly improved the activities of endogenous catalase and superoxide dismutase. Histopathological analysis of MNU-treated rats revealed disrupted colonic architecture characterized by distorted blood vessels, hyperchromasia, cellular pleomorphism, glandular atrophy, and lamina propria necrosis compared with normal and diet controls; these alterations were progressively ameliorated with higher levels of *O. gratissimum* leaf in the diet. Dietary inclusion of *O. gratissimum* increased the intake of crude fiber, polyphenols, and flavonoids. Crude fiber levels positively correlated with antioxidant enzyme activities (catalase and superoxide dismutase), while polyphenol and flavonoid contents correlated inversely with CEA. **Conclusion:** These findings suggest that *O. gratissimum* leaf exerts protective effects against MNU-induced colorectal injury, which may be associated with its dietary fiber and antioxidant polyphenolic content.

Introduction

Colorectal toxicity refers to structural damage resulting from drug- or chemical-induced injury to the large bowel, arising through defined pathogenic mechanisms such as direct mucosal cytotoxicity, impaired epithelial regeneration, or immune-mediated hypersensitivity. This

also includes inflammation, mucosal injury, and functional impairment of the large intestine. It is an emerging global health concern linked to environmental exposures, pharmaceuticals, and dietary and metabolic risk factors¹. Chronic colonic injury and inflammatory bowel disease are established drivers of colorectal carcinogenesis,

underscoring the clinical importance of preventing and mitigating early toxic damage to the colorectal mucosa. Although high-income settings have reported modest improvements in colorectal cancer outcomes through screening and better treatment, low- and middle-income regions continue to experience a rising burden of colorectal pathology associated with westernized diets, obesity, and metabolic disturbances that exacerbate mucosal inflammation and oxidative stress^{2,3}. Rapid urbanization and lifestyle changes, such as eating more red and processed meat, consuming less fiber and calcium, and having higher rates of obesity and diabetes, are predicted to increase vulnerability to colorectal injury and inflammation in Nigeria and other sub-Saharan African nations. This could have a negative impact on cancer risk and disease outcomes.

Conventional pharmacologic approaches for colitis and related toxicities can be limited by cost, access, and adverse effects, prompting growing interest in complementary dietary and phytotherapy strategies to protect the colonic epithelium^{5,6}. *Ocimum gratissimum* L, widely used as a culinary herb and a medicinal plant in West Africa, is rich in polyphenols, flavonoids, and essential oils that confer potent antioxidant and anti-inflammatory activities⁷. Methanolic and polyphenol-rich extracts of *O. gratissimum* have been shown in experimental colitis models to reduce disease activity, attenuate vascular permeability and granulocyte infiltration, lower colonic levels of Tumor Necrosis Factor- α (TNF- α), Interleukin-6 (IL-6), myeloperoxidase, nitric oxide, cyclooxygenase-2, and malondialdehyde, while restoring endogenous antioxidants such as superoxide dismutase, catalase, and glutathione. These effects translate into marked histological protection of the colonic mucosa, with reduced ulceration, edema, and tissue damage scores compared with untreated colitic controls and standard anti-inflammatory drugs⁸.

Similar phenolic-rich plant extracts from other species have demonstrated parallel benefits in experimental colitis, supporting a broader role for dietary phytochemicals in maintaining intestinal barrier integrity and limiting colorectal toxicity⁹.

Given the rising exposure to dietary and metabolic risk factors in Nigeria and the strong preclinical evidence that *O. gratissimum* mitigates colitis-related oxidative stress and inflammation, further investigation of its efficacy as a dietary intervention to prevent colorectal toxicity is warranted. This study evaluated whether dietary inclusion of graded levels of *O. gratissimum* leaf attenuates MNU-induced oxidative stress and histopathological alterations in the colon of male Wistar rats.

MATERIALS AND METHODS

Materials

Fresh leaves of *O. gratissimum* (*Scent leaf*) were collected from Angwan Jeba farm, Zaria, Northwestern Nigeria and identified in the herbarium section of the Department of Botany, Ahmadu Bello University with a voucher number 1285. The leaves were sorted and air dried at room temperature (25°C). The dried leaves were pulverized to powder using an automated grinding machine at Institute of Agricultural Research, Ahmadu Bello University, Zaria. The powdered leaves were then stored in an air-tight container prior to the supplemental formulation

Animal handling

Male Wistar rats of 5 weeks old weighing 70-90g were purchased and housed in the Faculty of Pharmaceutical Sciences Animal House, Ahmadu Bello University Zaria and allowed free access to water and standard diet for two weeks for acclimatization before commencement of experiment. The study was conducted in accordance with the US guideline as contained in the National Institute of Health guide for the care and use of laboratory animals¹⁰. The rats were weighed using (AWS Sc-2kg digital Scale 2000g x 0.1g Jewelry Gold Silver, USA) and randomly divided into six (6) groups of five animals each.

Experimental diets

Growers mash from Vital feed (Jos, Plateau state Nigeria) was used in the entire study. The experimental diet was mixed with the dried powdered *Ocimum gratissimum* leaf using a blender to ensure uniform mixing of the components. All supplemented diets were molded into balls and dried under the sun and were fed to the experimental groups according to the percentage of inclusion (0%, 2.5%, 5% and 10%) following a method¹¹.

Methods

Experimental design

This experiment was designed to determine the preventive effect of dietary inclusion of *Ocimum gratissimum* on MNU-induced oxidative stress and colonic mucosal injury in male Wistar rats. The animals were fed either a basal diet or a diet containing their group's assigned percentage of *O. gratissimum* for eight (8) weeks before MNU exposure. Subsequently, four (4) groups received intrarectal MNU at 6 mg/kg body weight for 10 weeks, while two (2) control groups received normal saline for ten (10) weeks. All animals continued on their respective diets throughout this period. The animals were maintained on the control or

experimental diets until the end of the experiment

Animal Sacrifice and Tissue Homogenization

The animals were anaesthetized with 83mg ketamine/kg and 13mg xylazine/kg via intraperitoneal route and euthanized by decapitation one week after completion of the induction period. The rats were subsequently dissected, and the colon, kidneys, and liver were excised and fixed in 10% formal saline for histological analysis. The organs were excised and weighed, and the relative organ weights were calculated. A portion of the liver and one kidney from each rat were immediately rinsed with ice-cold normal saline. A 10% (w/v) tissue homogenate was prepared by homogenizing 100 mg of tissue in 1 mL of 50 mM phosphate buffer (pH 7.4) using a mortar and pestle. The homogenates were centrifuged at 1000 rpm for 10 min using a Heraeus Labofuge 300 centrifuge (Thermo Fisher Scientific Inc., USA). The resulting supernatants were collected and stored at -20 °C until analysis. The supernatants were used to determine the activities of endogenous antioxidant enzymes, including catalase (CAT) and superoxide dismutase (SOD), and the level of lipid peroxidation, assessed using the thiobarbituric acid-reactive substances (TBARS) assay. Whole blood samples were collected into EDTA bottle and used for haematological analysis, while serum was collected from another blood sample was allowed to coagulate at room temperature and stored at -20°C for analysis of carcinoembryonic antigen (CEA).

Biochemical analysis

Lipid peroxidation in the liver and kidney homogenates was measured via spectrophotometry according to the method described by¹². Briefly, 50 µL of the tissue homogenate supernatant was deproteinized with 1 mL of 14% trichloroacetic acid (TCA), followed by the addition of 1 mL of 0.6% thiobarbituric acid (TBA). The mixture was heated at 80 °C for 30 min and subsequently cooled on ice for 5 min. The samples were then centrifuged at $2,000 \times g$ for 10 min, and the absorbance of the resulting supernatant was measured at 535 nm using a spectrophotometer. TBARS concentration was calculated using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$. Total protein was determined by Lowry et al¹³. Catalase activity determined by method of Aebi¹⁴, while superoxide dismutase activity was determined using the method described by Martin et al¹⁵.

Extraction and estimation of total phenolic content

Ten millilitres (10 mL) of 70% methanol was introduced into a glass test-tube containing 0.1g of the powdered

sample. This was followed by heating in a water bath for 2 h at 37 °C with shaking every 15 min. The mixture was then allowed to cool to room temperature before being centrifuged at 3000 rpm for 10 min. The supernatant was collected for total flavonoids determination. Absolute methanol was used for the extraction of total phenolic content. The Folin-Ciocalteu method was used to determine the total phenolic content¹⁶. Briefly, 100 µl of each diluted extract were mixed with 2.8 ml of deionized water and 2 ml of 50% Folin Ciocalteu's phenol reagent. The mixture was incubated for 30 min at room temperature, and absorbance was taken at 765 nm using a spectrophotometer. The result was expressed as milligram gallic acid equivalent per gram extract (mg GAE/g extract).

Determination of total flavonoid content

The total flavonoid content was determined using the aluminum chloride colorimetric method described by Chang et al¹⁷. Briefly, 100µl of each extract were mixed with 1.5 ml of 95% ethanol, 100 µl of 1 M potassium acetate and 2.8 ml of deionized water and the absorbance of the reaction mixture were determined at 415 nm after incubation at 45°C for 2 h. The total flavonoid content was expressed as milligram quercetin equivalent per gram extract (mg QE/g extract).

Crude Fibre Determination

Two grams (2 g) of the sample was weighed and transferred into a 1 L conical flask. One hundred milliliters (100 ml) of sulfuric acid (0.255 mol/L) was heated to boiling and then introduced into the conical flask containing the sample. The mixture was boiled for 30 min, ensuring that the level of the acid was maintained by the addition of distilled water. After 30 min, the mixtures were filtered through a muslin cloth supported by a funnel. The residue was rinsed thoroughly until its washing was no longer acidic to litmus. The residue was then transferred into a conical flask and 100 ml of sodium hydroxide (0.313 mol/L) was then brought to boil and then introduced into the conical flask containing the sample. The contents were then boiled for 30 min, ensuring that the level of the acid was maintained by the addition of distilled water. After 30 min, the contents were then filtered through a muslin cloth supported by a funnel. The residue was rinsed thoroughly until its washing was no longer alkaline to litmus. The washed residue was transferred into a pre-weighed, dried crucible and incinerated in a muffle furnace at 600 °C until a constant weight was obtained. The crude fibre content was then calculated according to the method described by¹⁸.

Determination of CEA:

Serum CEA levels were determined using an assay kit procured from Diagnostic Automation/cortex Diagnostics (DACD) Incorporation, Calabasas California USA, following the principle of Enzyme Linked immunosorbent assay (ELISA). Serum samples collected from the experimental rats were loaded into wells of a pre-coated CEA biotin-conjugated antibodies microplate. This was followed with a solution containing, avidin-conjugated to horseradish peroxidase (HRP). The mixture in the wells was incubated for 5 min. After the specified incubation period, 3,3',5,5'-tetramethylbenzidine (TMB) substrate was added, resulting in the development of a coloured reaction. The absorbance was measured at 450 nm using a microplate reader, and the serum CEA concentrations were determined from the calibration curve provided with the assay kit..

Histopathology:

The colon, liver, and kidney tissues were rapidly excised and fixed in 10% formal saline for 18–24 h. The fixed tissues were subsequently dehydrated through a graded series of ethanol concentrations (70%, 80%, 90%, 95%, and absolute ethanol), with each concentration maintained for 2 h, following the method described by Kiernan [19]. The tissues were cleared in xylene and subsequently infiltrated with molten paraffin wax. Briefly, the tissues were transferred into molten paraffin wax in an oven maintained at 65 °C for 1 h and then transferred into a second container of molten paraffin wax for a further 2 h. The tissues were subsequently embedded in moulds containing molten paraffin wax and allowed to solidify.

Sections approximately 3 µm thick were prepared using a microtome and mounted on clean glass slides. The sections were stained with haematoxylin and eosin (H&E) using standard histological techniques. The stained sections were examined under a light microscope for histopathological changes, and photomicrographs were taken at appropriate magnifications.

Correlation Coefficient determination

Dietary components (flavonoid, crude fibre, polyphenol), colorectal biomarkers (CEA), oxidative stress markers (MDA), and antioxidant enzymes (catalase, SOD).where analyzed to estimate their level of correlation with a two tail significance level (0.01 and 0.005) and a perfect correlation being 1. Values with Positive sign show correlation in parameters while negative shows inverse correlation in parameters.

Statistical Analysis

The statistical significance between the control and other groups of experimental animals were determined by one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc multiple comparisons. The results are presented as mean ± SEM at confidence level of 95% ($p \leq 0.05$)

RESULTS

3.0 RESULTS

3.1 Effect of *O. gratissimum* leaf inclusion diets on the level of carcinoembryonic antigen (CEA) of MNU-induced colorectal toxicity rats

The result obtained for the levels of carcinoembryonic antigen (CEA) in rats treated with MNU and administered with *O. gratissimum* leaf supplementation are presented in Figure 1. There was a significant increase ($P < 0.05$) in the levels of CEA in the MNU-treated control group (3.47 ± 0.29) compared to the normal control and the varying supplemented diet groups. In general, the level of CEA in the supplemented diet groups decreased with increasing level of dietary supplementation. However, there was no significant difference ($P < 0.05$) between the normal control group and 10% *O. gratissimum* inclusion control (Figure 1).

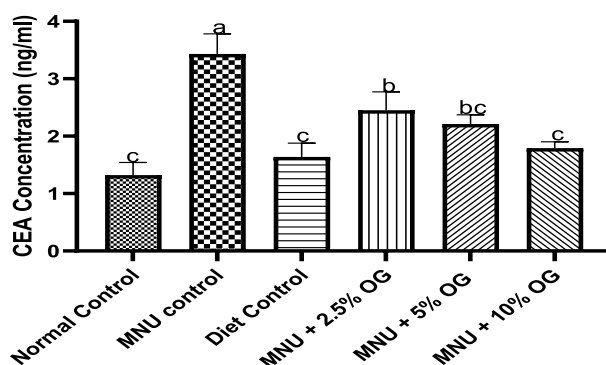


Figure 1: Serum CEA Levels in MNU-Treated Rats Fed Diets Supplemented with Different Levels of *Ocimum gratissimum*. Values are expressed as Mean ± S. E. M, bars with different alphabets are statistically significant ($p < 0.05$), $n = 5$

3.2. Quantitative Analysis of Total Phenolic and Flavonoid Contents of Diets Supplemented with *Ocimum gratissimum* Leaf.

The total phenolic, total flavonoid, and dietary fiber contents of *O. gratissimum* leaf and the supplemented diets are presented in Table 1. The pure *O. gratissimum* leaf contained the highest levels of total phenolics, total flavonoids, and dietary fibre, whereas the basal diet had the lowest values. Among the supplemented diets, the contents of total phenolics, total flavonoids, and dietary fibre increased progressively with increasing levels of *O. gratissimum* leaf inclusion. (Table 1).

Table 1: Total polyphenolic, total flavonoid and dietary fiber contents of the leaf of *Ocimum gratissimum* supplemented diets

Level of Inclusion	Total Polyphenols (mg GAE/g extract)	Total Flavonoid (mg QE/g extract)	Dietary fiber (%)
0% Inclusion (Basal feed)	59.32 ± 0.003	30.97± 0.0100	7.49± 0.210
2.5% Inclusion	62.69 ± 0.880	34.16 ± 0.0067	8.23± 0.320
5% Inclusion	78.62 ± 0.978	59.71 ± 2.5390	8.34± 0.410
10% Inclusion	107.93 ± 5.770	78.26 ± 6.6380	8.55± 0.310
<i>Ocimum gratissimum</i> Leaf	165.45 ± 3.290	124.62±6.2904	9.07±0.010

Values are Mean ± SEM

3.3 Effects of Graded Dietary Inclusion of *Ocimum gratissimum* Leaf on N-Methyl-N-Nitrosourea-Induced Colorectal Toxicity in Male Wistar Rats Wistar

As shown in Table 2, MNU treatment significantly increased colonic MDA levels compared with the normal control group ($p < 0.05$), indicating increased lipid peroxidation. Dietary supplementation with *O. gratissimum* leaf resulted in a progressive reduction in MDA levels with increasing inclusion levels. Conversely, CAT and SOD activities increased progressively with increasing levels of *O. gratissimum* supplementation Wistar (Table 2).

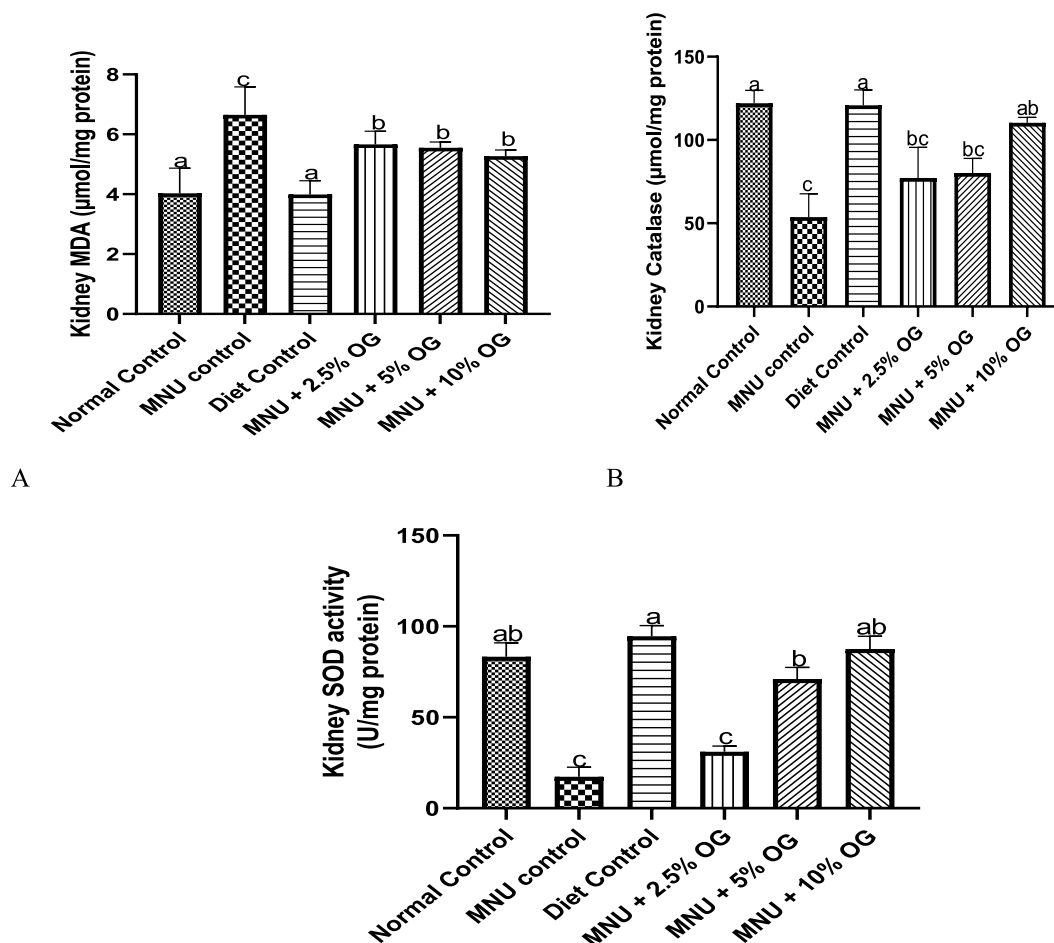
Table 2: The effect of dietary inclusion with leaf of *O. gratissimum* on malondialdehyde (MDA) , catalase and superoxide dismutase on the colon of MNU-treated Wistar rats

TREATMENT	MDA ($\mu\text{mol/g}$ tissue)	Catalase ($\mu\text{mol/g}$ tissue)	Superoxide dismutase (U/mg protein)
Control feed	84.03 ± 0.84 ^b	71.98±7.7 ^a	42.22 ± 7.6 ^{ab}
MNU + Basal Feed	109.65 ± 0.93 ^a	23.62±3.9 ^c	16.14 ± 1.3 ^c
10% O.C Control	83.99 ± 0.46 ^b	70.74 ± 9.2 ^a	51.49 ± 5.8 ^b
MNU + 2.5% O.G diet	105.66 ± 0.44 ^{ab}	32.13 ± 8.3 ^{bc}	19.98 ± 3.2 ^c
MNU + 5% O.G diet	95.55 ± 0.19 ^{ab}	40.13 ±15.7 ^{bc}	39.98 ± 6.4 ^a
MNU + 10% O.G diet	85.27 ± 0.21 ^{ab}	60.21 ± 13.2 ^b	46.49 ± 7.1 ^{ab}

Values with different superscripts down the column are significantly different ($P < 0.05$)

3.4 Effect of *Ocimum gratissimum* diet inclusion on the kidney oxidative status of N-methyl-N-nitrosourea-induced colorectal toxicity in male Wistar rats

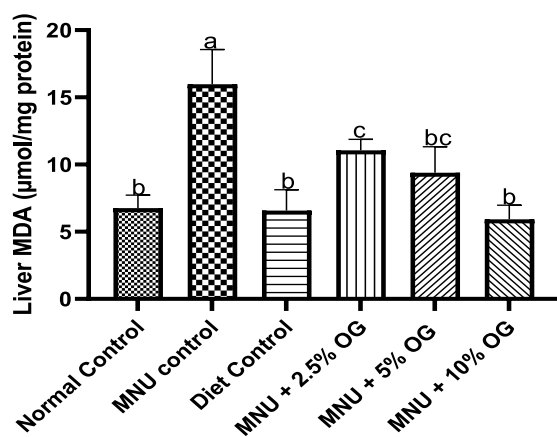
Dietary supplementation with *Ocimum gratissimum* leaf modulated kidney oxidative stress markers in MNU-treated Wistar rats. The MNU-treated control group showed elevated kidney MDA levels alongside reduced SOD and catalase activities compared with the normal control and 10% *Ocimum gratissimum* supplemented control groups. In MNU-treated rats fed diets supplemented with *Ocimum gratissimum* leaf, kidney MDA levels decreased progressively with increasing levels of leaf inclusion, whereas SOD and catalase activities increased with increasing levels of supplementation. These changes suggest that (Figure 2).



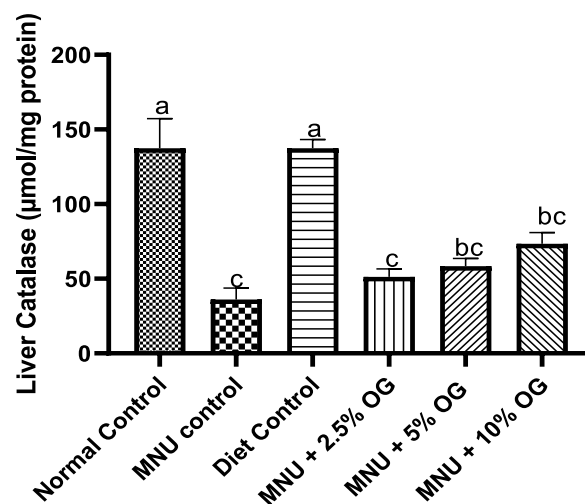
Figures 2A-C: Effects of *Ocimum gratissimum* (OG) diet inclusion on Kidney MDA, CAT and SOD levels of Wistar rats administered MNU. Values are expressed as Mean $\pm$ S. E. M, bars with different alphabets are statistically significant ($p < 0.05$), $n = 5$

3.5 Effects of Graded Dietary Inclusion of *Ocimum gratissimum* Leaf on Liver Oxidative Status in N-Methyl-N-Nitrosourea-Induced Colorectal Toxicity in Male Wistar Rats

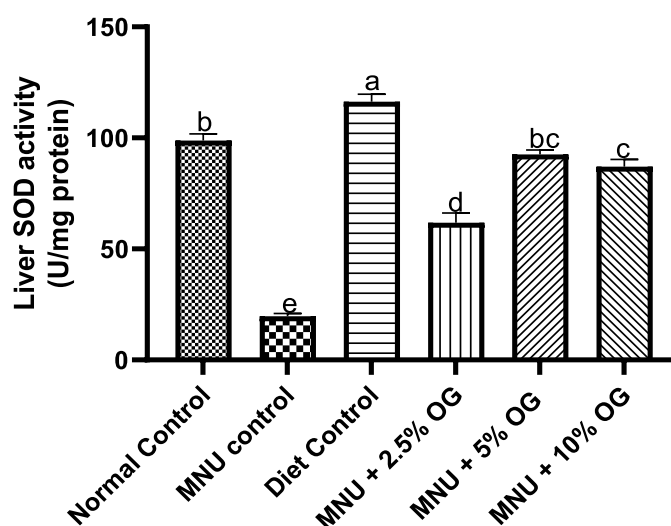
A comparable pattern as seen both in the colon and kidney was observed in the liver. MNU administration resulted in increased hepatic MDA levels and a reduction in SOD and catalase activities relative to the normal control and 10% *Ocimum gratissimum*-supplemented groups, reflecting pronounced oxidative insult. However, dietary *Ocimum gratissimum* supplementation in MNU-treated rats led to a graded reduction in liver MDA with rising levels of the leaf in the diet, accompanied by a corresponding elevation in hepatic SOD and catalase activities. This dose-dependent response suggests that *Ocimum gratissimum* leaves exerts protective effects against MNU-induced hepatic oxidative stress by limiting lipid peroxidation and reinforcing the antioxidant defense system (Figure 3).



A



B



Figures 3A-C: Effects of *Ocimum gratissimum* (OG) diet inclusion Liver MDA, SOD, and CAT activities of Wistar rats administered MNU. Values are expressed as Mean $\pm$ S. E. M, bars with different alphabets are statistically significant ($p < 0.05$), $n = 5$.

3.6 Protective Effects of Dietary *Ocimum gratissimum* Leaf against MNU-Induced Colorectal Histopathological Alterations in Male Wistar Rats.

The MNU-treated control group exhibited marked histopathological alterations, including distorted colonic architecture, vascular distortion, hyperchromatic nuclei, cellular pleomorphism, anisocytosis, poikilocytosis, glandular atrophy, and necrosis of the lamina propria (Figure 4b) compared with the normal control (Figure 4a). These histopathological alterations were progressively attenuated with increasing levels of dietary *O. gratissimum* leaf inclusion, with improved preservation of colonic architecture observed in the supplemented groups (Figure 4d–f).



Figure 4a: NORMAL CONTROL (H&E;x 200)
The normal control group with well-arranged architecture of the colon without atrophy of the gland and a living lamina propria.

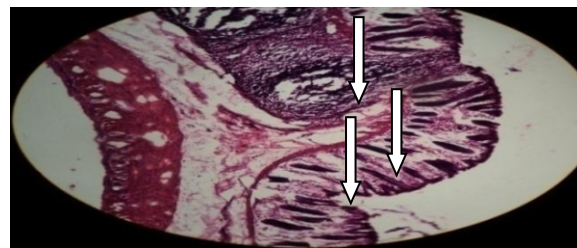


Figure 4b: MNU CONTROL (H&E; x 200)
A colonic architecture with distorted blood vessels, hyper chromaticity, pleomorphism, atrophy in the gland and necrosis of the lamina propria.

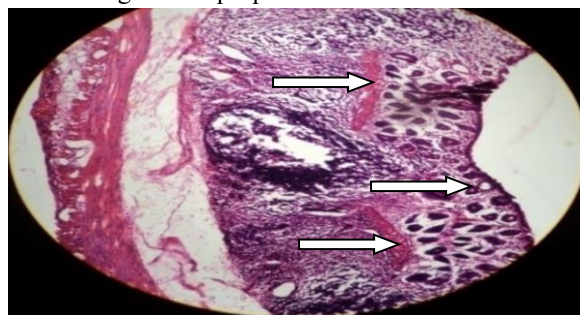


Figure 4c: MNU + 2.5% *O. gratissimum* (H&E; x 200)
A distorted architecture of the colon and a fiber connecting tissue around the intestinal gland as a preventive mechanism to protect the gland.

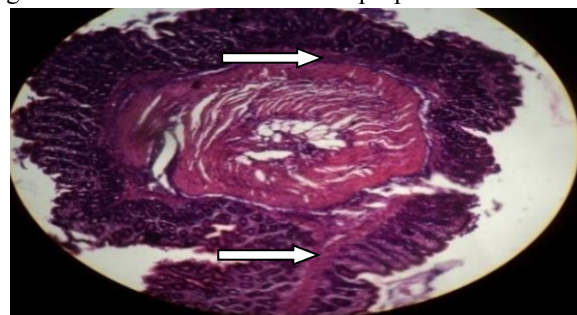


Figure 4d: MNU + 5% *O. gratissimum* (H&E; x 200)
An observable repair in the architecture of the colon with living lamina propria and fiber connective tissue being around the intestinal gland (H&Ex200)

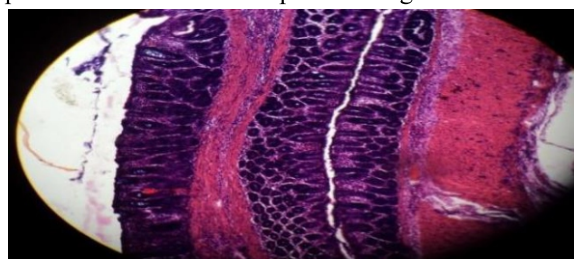


Figure 4e: MNU + 10% *O. gratissimum* (H&E; x 200)
Normal architecture of the colon with well-arranged lamina propria, no necrosis, well organized intestinal gland (H&E; x 200)

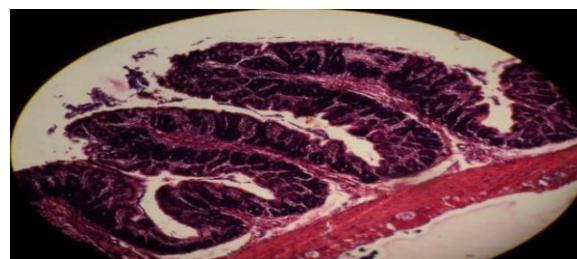


Figure 4f: 10% *O. gratissimum* diet control (H&E; x 200)
A well-arranged architecture of the colon and crypt.

Figures 4a-f: Histological Features of the Colon of N-Methyl-N-Nitrosourea-Treated Rats Fed Diets Supplemented with Graded Levels of *Ocimum gratissimum* Leaf

3.7 Correlation between *Ocimum gratissimum* Leaf Inclusion and Colorectal Biomarkers and Oxidative Stress Parameters (MDA, CAT and SOD) The correlation table shows the strength and direction of relationships between dietary components (flavonoid, crude fibre, polyphenol), colorectal biomarkers (CEA), oxidative stress markers (MDA), and antioxidant enzymes (catalase, SOD). Values near ± 1 indicate strong correlations; negative values indicate inverse relationships. CEA strongly correlates with MDA (positive) and antioxidant enzymes (negative).

Table 3: Correlation Coefficients among Biomarkers and Antioxidant Parameters in Rats Fed Diets Supplemented with Graded Levels of *Ocimum gratissimum* Leaf

	CEA	MDA	CATALASE	SOD
Flavonoid	-0.233	-0.623	0.471	0.796
Crude Fibre	0.180	-0.261	0.160	0.424
Polyphenol	-0.303	-0.641	0.471	0.786
CEA	1	0.884	-0.938	-0.808

Pearson correlation with Venue are expressed in Mean values (N=5), CEA: Carcinoembryonic antigen, MDA: Malondialdehyde SOD: superoxide dismutase

Discussion

Colorectal toxicity leading to colorectal cancer remains a significant global health challenge, with a disproportionate burden in low- and middle-income countries such as Nigeria, where late-stage diagnosis and limited access to effective therapies contribute to poor outcomes^{20,21}. In response, there is growing scientific interest in the chemopreventive and therapeutic potential of medicinal plants, particularly those rich in dietary fiber and antioxidant phytochemicals, as adjuncts or alternatives to conventional treatments²². The observed associations between colonic biomarkers, oxidative stress parameters, and the antioxidant and dietary fibre content of *Ocimum gratissimum* suggest that the plant may have potential as a complementary dietary intervention for supporting colorectal health. However, further studies are required to establish the underlying mechanisms and clinical relevance of these findings²³. Hence, this study provides a possible mechanism underlining the preventing potentials of graded levels of *Ocimum gratissimum* dietary inclusion in N-methyl-N-nitrosourea-induced oxidative stress and colonic mucosa injuries in male Wistar rats. Carcinoembryonic antigen (CEA) of colorectal toxicity is the most frequently used tumor biomarker to monitor patients with colorectal cancer for early reoccurrences because CEA is usually present only at very low levels but concentrations are raised in colorectal cancer²⁴. The significant increase ($p < 0.05$) in serum CEA levels observed in the MNU-treated control group compared with the normal control group was significantly attenuated following dietary inclusion of *Ocimum gratissimum* leaf. This finding may indicate a

potential chemopreventive effect of *O. gratissimum* leaf, possibly attributable to its bioactive constituents. However, further studies are required to establish the specific constituents and mechanisms responsible for this effect. Elevated levels of reactive oxygen species (ROS) have been identified in almost all cancers, where they promote many aspects of tumor development progression²⁵. This agrees with the result of the MDA presented in (Table 2) which showed elevated lipid peroxidation in the colon of the MNU-treated rats particularly in the MNU-treated control group when compared with the normal control group. The increased lipid peroxidation may be as a result of cellular membrane degeneration and DNA damage due to singlet oxygen production²⁶. The body counteracts oxidative stress through endogenous antioxidant defence mechanisms, particularly antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), which contribute to the neutralization of ROS and limitation of oxidative damage. The observed reduction in MDA levels accompanied by increased SOD and CAT activities following *O. gratissimum* supplementation therefore suggests that the plant may enhance antioxidant defence and attenuate MNU-induced oxidative stress. Reduced SOD activity in cancer cells may render malignant cells highly dependent on SOD for survival.²⁷ This aligns with the results in Table 2, which showed lower SOD activity in the MNU control group compared with the normal control group. This reduction may result from the suppressive potential of the induced carcinogen, enabling it to exert its maximum effect. In contrast, groups treated with different dietary inclusions showed elevated SOD activity in a dose-

dependent manner, possibly due to exogenous antioxidants in the diet that complement endogenous antioxidants in combating cancer cells.²⁸ A decrease in the activity of catalase was observed in the MNU-treated groups when compared to the normal control group. This decrease agrees with the report of Gal *et al*.²⁹ who observed a significant decrease ($p < 0.05$) in catalase activity in cancer tissue as a result of increased oxidative stress coupled with membrane damage due to lipid peroxidation antioxidant enzymes. However, dietary inclusion of *Ocimum gratissimum* leaf resulted in a progressive increase in catalase activity with increasing levels of supplementation. This finding suggests that *O. gratissimum* may enhance endogenous antioxidant defence and thereby contribute to the attenuation of oxidative stress. The effect may be partly attributable to the polyphenolic and flavonoid constituents of the plant, which possess antioxidant and potential chemopreventive properties. These exogenous antioxidants have been reported to play a protective role via acting as antitumor agents through various mechanisms one of which is the reduction of oxidative stress. Other mechanisms could be via cellular growth arrest through the induction of ROS-dependent premature senescence, inhibition of angiogenesis and down regulation of selective activation of cancer receptors³⁰.

Histopathological findings were consistent with the changes in the levels of carcinoembryonic antigen. The MNU-control group and the group fed with the lowest supplemented diet group showed distorted colonic architecture with extensive necrosis of the *lamina propria*, as well as cell proliferation and hyperchromasia.^{31,32} Additionally, colonic mucosa showed cellular pleomorphism and atrophy of glands in plates 1b, 1c, which are biomarkers for colon carcinogenesis³³. However, MNU-treated groups receiving the highest level of *O. gratissimum* leaf inclusion and the diet control group showed no apparent histopathological alterations compared with the normal control group.

Ugbogu *et al*.³⁴ conducted a pharmacognostical evaluation of *Ocimum gratissimum* as a traditional medicine for various diseases. Furthermore, a recent study by Ikokide *et al*.³⁵ on the phytochemicals of this plant revealed high levels of polyphenols, flavonoids, and other phytochemical constituents. The results presented in Table 1 confirmed that *Ocimum gratissimum* is indeed a rich source of polyphenols and flavonoids. Consequently, it was not surprising that diets supplemented with this plant produced a significant chemopreventive effect on colon toxicity, given the proposal that foods containing multiple antioxidant phytochemicals are far more effective for cancer prevention than a single chemical compound in

foods.³⁶ These phytochemicals exert their effects by blocking the initiation of carcinogenesis through the inactivation of exogenous or endogenous genotoxic molecules, including reactive oxygen species (ROS); in addition, they inhibit the activity and synthesis of carcinogen-metabolizing enzymes.³⁷ Alongside polyphenols and flavonoids, dietary fiber was highest in the 10% *Ocimum gratissimum* supplemented diet, which caused the greatest reduction in CEA levels (Figure 1) and in the histopathological changes of the colon (Figure 4c, 4d, 4e). Possible mechanisms by which fibers may inhibit colon toxicity include the dilution and adsorption of carcinogens and/or promoters within the intestinal lumen, the modulation of colonic microbial metabolic activity, and the biological modification of intestinal epithelial cells.³⁸ Dietary fibre may contribute to protection against colorectal carcinogenesis through several mechanisms, including increased faecal bulk, dilution and binding of potentially harmful luminal compounds, modulation of intestinal transit, and alteration of the colonic microbial environment³⁹. Dietary fibre comprises a diverse group of non-digestible food components, including non-starch polysaccharides, lignin, and resistant or analogous polysaccharides. These components can increase faecal bulk and, depending on their physicochemical properties, influence intestinal viscosity and transit time. By increasing faecal bulk and reducing the concentration and contact time of potentially harmful compounds with the colonic mucosa, dietary fibre may reduce exposure of epithelial cells to carcinogenic substances⁴⁰. Dietary fiber could bind and excrete potential luminal carcinogen such as secondary bile acids and lower fecal pH in the colon³⁸. It is also associated with phytochemicals such as phenolics, lignans, beta-glucan and inulin⁴¹. For example, arabinoxylan, a constituent of hemicellulose is an important source of phenolic compounds that may be released in the colon during fermentation of complexed fibres⁴². These bioactive substances may protect the GI tract from oxidative damage as the dietary fiber source is much higher in the colonic lumen than in plasma and other tissue thus, delaying the onset of colonic mucosa injuries.

the strength and direction of relationships between dietary components (flavonoid, crude fibre, polyphenol), colorectal biomarkers (CEA), oxidative stress markers (MDA), and antioxidant enzymes (catalase, SOD) showed that CEA strongly correlates with MDA (positive) and antioxidant enzymes (negative), which confirm the positive role of these constituents and the protective potential of *Ocimum gratissimum* leaf in preventing colon toxicity. Therefore, it can be concluded that *Ocimum gratissimum* leaf being a rich source of polyphenols, flavonoids and

dietary fibre can help chemo-prevent and attenuate colon carcinogenesis, when used in the right proportions in diets.

Conclusion

In conclusion, *Ocimum gratissimum* leaf demonstrated potential chemopreventive effects against MNU-induced colorectal toxicity, as evidenced by reduced CEA levels, improved antioxidant status, and preservation of colonic architecture. These effects may be associated with its dietary fibre and phytochemical constituents.

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Consent to publish

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