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RESEARCH ARTICLE

Eur J Biol 2021; 80(2): 119-128

Therapeutic Effects of *Momordica charantia* L. Ethanolic Extract on Acetic Acid-Induced Ulcerative Colitis in Rats

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Please cite this article as: Ozbeyli D, Sen A, Aykac A, Terali K, Cilingir-Kaya OT, Senkardes I, Sener G. Therapeutic Effects of *Momordica charantia* L. Ethanolic Extract on Acetic Acid-Induced Ulcerative Colitis in Rats. Eur J Biol 2021; 80(2): 119-128. DOI: 10.26650/EurJBiol.2021.1016222

ABSTRACT

Objective: This study aims to investigate the effect of *Momordica charantia* L. (MoC) ethanolic extract on ulcerative colitis (UC) and was explored *in vitro* and *in vivo*.

Materials and Methods: The rats were divided into control (C), saline-treated colitis (AA), MoC extract-treated colitis (AA+MoC), and sulfasalazine (SS)-treated colitis (AA+SS) groups. Colitis was induced by acetic acid. MoC extract, SS or saline were given to the related groups for 3 days. Interleukine-1β, malondialdehyde, glutathione levels, myeloperoxidase activity, bax/bcl-2 ratio, caspase-9 and caspase-3 levels were measured in colon and macroscopic and histopathologic examinations were done. Total phenolic/flavonoid content and biological activity of MoC were evaluated by *in vitro* analysis.

Results: Compared to the control group, with acetic acid application interleukin-1β levels, myeloperoxidase activity, malondialdehyde levels, bax/bcl-2 ratio, caspase-9 and caspase-3 levels were significantly upregulated, while glutathione levels were significantly decreased in the AA group. In contrast, MoC and SS treatments reduced interleukin-1β, malondialdehyde levels, myeloperoxidase activity, bax/bcl-2 ratio, and caspase-9 and caspase-3 levels. Glutathione levels increased upon MoC or SS treatment. Increased macroscopic and microscopic scoring with AA improved with MoC or SS treatment, but the MoC or SS treated groups had higher score values than the control. Also, *in vitro* results showed that MoC exhibited 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical scavenging activity as well as significant anti-lipoxygenase activity. In addition, MoC extract showed a potent anti-inflammatory activity compared to standard indomethacin.

Conclusion: Our biochemical, *in vitro* and histopathologic analysis indicate that MoC is likely to prove beneficial in UC therapy.

Keywords: *Momordica charantia* L., ulcerative colitis, apoptosis, oxidative stress, radical scavenging activity, anti-lipoxygenase activity

INTRODUCTION

Ulcerative colitis (UC) affects millions of people worldwide and is characterized by recurrent mucosal inflammation and ulceration of the large intestine (1). Although the exact aetiology of UC is not well documented, it involves a dysregulated immune system as well as environmental effects. Current UC treatments are based on the suppression of inflammation (2). The

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Submitted: 30.10.2021 • **Revision Requested:** 09.11.2021 • **Last Revision Received:** 15.11.2021 • **Accepted:** 16.11.2021 • **Published Online:** 06.12.2021

acetic acid (AA)-induced UC model represents a good model for clinical, biochemical, and histological examinations in colonic inflammation (3). AA causes major epithelial damage and is characterized by increased leukocyte infiltration, increased inflammatory mediators, vascular dilatation, oedema, and large, massive ulceration of the non-transmural large intestine (4). Several cytokines are involved in the pathogenesis of UC, including tumour necrosis factor (TNF)-α and interleukin1β (IL-1β) (5). Oxidative stress plays a significant role in the aetiology of UC in such a way that reactive oxygen species (ROS) formation during inflammation causes apoptosis-mediated cellular death and loss of epithelial cells. However, an increase in the apoptotic cells leads to a deterioration of the epithelial defence in the colon and accelerates mucosal inflammation (6).

Current therapies mainly aim at decreasing inflammation using anti-inflammatory drugs as well as at reducing oxidative stress (3). Aminosalicylates, corticosteroids, and immunosuppressants that are used in the treatment of mild and moderate UC provide regression of disease symptoms. One of them, sulfasalazine (SS), which is utilized as standard therapy in the disease, corresponds to an aminosalicylate derivative. The 5-aminosalicylic acid component of SS is liable for its therapeutic effect, and the majority of its side effects are linked to the sulphapyridine portion (5). However, treatments using these agents have drug-related side effects that can lead to some problems (7). On the other hand, natural plants have long been used to treat various diseases and to avoid the side effects of drugs. *Momordica charantia* L. (MoC), also known as bitter gourd, bitter apple, or bitter melon, is a green to yellow-coloured flowering plant and a member of the Cucurbitaceae family. MoC widely grows in Asia and the Mediterranean area, and the main phytochemical ingredients of MoC include essentially flavonoids, phenolic acids, cucurbitane-type triterpenoid saponins (momordicine, momordin, and momordicoside (8-10). Standard constituents of MoC are charantin (a mixture of 5. 25-stigmasteryl glucoside and β-sitosteryl glucoside) and momordicine, which are steroidal saponins (8-11). Momordicine and charantin are mainly responsible for the beneficial activities and bitterness of MoC (8-12). MoC has been reported to have more biological effects such as antioxidant, anti-apoptotic and anti-inflammatory (9, 12, 13). In addition, MoC fruit is used externally for the quick healing of abrasions and internally for the cure of peptic ulcers in Turkish folk medicine (14). MoC has been shown to alleviate dextran sulfate sodium (DSS)-induced colitis in mice by depressing inflammatory cytokines and increasing regulatory T cells (15). Additionally, MoC has been demonstrated to have significant anti-inflammatory effects on trinitrobenzene (TNBS)-induced colitis that mimics Crohn's disease by reducing myeloperoxidase (MPO) activity and cytokine levels in rats (10).

During colitis induction, administered MoC therapy can improve colonic damage ameliorated by decreased colonic oxidative stress and apoptosis. Therefore, the present study was designed to assess the potential antioxidant, anti-inflammatory and anti-apoptotic effects of MoC on AA-induced ulcerative rat models through *in vitro* and *in vivo* analysis.

MATERIALS AND METHODS

Chemicals

Acetic acid, dimethyl sulfoxide (DMSO), 2-(4-(2-hydroxyethyl) piperazin-1-yl) ethanesulfonic acid (HEPES), hexadecyltrimethylammonium bromide (HETAB), N-(2-Hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid), trichloroacetic acid (TCA), thiobarbituric acid (TBA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), o-Dianisidine, $Na_2HPO_4.2H_2O$, K_2HPO_4, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulfate, indomethacine, ascorbic acid, trolox, lipoxidase from Glycine max (soybean), Folin-Ciocalteu reagent, linoleic acid, formaldehyde, dithiothreitol (DTT), glycerol, Tris-HCl, Ethylenediaminetetraacetic acid (EDTA), and Triton X-100, $NaNO_2$, $AlCl_3{\cdot}6H_2O$, NaOH, methanol, and ethanol were supplied by Sigma (Sigma-Aldrich, St. Louis, MO, USA). All antibodies for immunoblotting were purchased from Santa Cruz Biotechnology Inc (Santa Cruz, CA, USA). Sodium pentobarbital was purchased from IE Ulagay (Istanbul, Turkey). All other chemicals were of the purest grade commercially available.

MoC Extract Preparation

MoC fruits were collected from the rural district of Gemlik in Bursa, Turkey in August 2019 and identified by Dr. İsmail Şenkardeş from the Department of Pharmaceutical Botany, Faculty of Pharmacy, Marmara University. The voucher specimens were deposited in the Herbarium Faculty of Pharmacy, Marmara University (Marmara No: 22446). Fresh fruit with seeds was blended and then were macerated by 95% ethanol (1000 mL) for 48 h. The extraction process was repeated until the solvent became colourless. The filtrate was evaporated and concentrated at 40°C. The obtained dried ethanol extract of MoC was stored at +4°C until further analysis.

In Vitro Antioxidant and Anti-inflammatory Activity of MoC

The DPPH radical scavenging capacity measurement of MoC extract was performed in line with a previously reported procedure by Zou et al. (16). In short, 10 µL of extract or standard ascorbic acid in DMSO at different concentrations were mixed with 190 µL of 0.1 mM DPPH solution in methanol in a 96-well plate. Mixtures were left to incubate for 30 min in the dark at room temperature. Then, the absorbance was taken at 517 nm. Tests were carried out in triplicate.

The radical scavenging activity percentage of extracts and compounds against DPPH radicals were calculated according to the following: DPPH radical-scavenging activity (%) = $[(A_0\text{-}A_1)/A_0] \times 100$, where A_0 is the absorbance of the control (containing all reagents except the test extracts/compounds) and A_1 is the absorbance of the extracts/compounds. The extract (or standard) concentration resulting in a 50% inhibition (IC_{50}) was calculated by regression equations (by plotting the extract/standard solution concentration versus percentage of inhibition). Lower IC50 values indicate higher inhibitory potential of the tested extract (16).

ABTS radical cation scavenging activity was tested according to Zou et al. (16). ABTS radical cations were produced by mixing

equal volumes of ABTS (7 mM in H_2O) and potassium persulfate (4.9 mM in H_2O) and allowing them to react for 12-16 h at room temperature in the dark. The ABTS radical solution was then diluted with 96% ethanol to obtain an absorbance of about 0.7 at 734 nm using a spectrophotometer. 10 µL of extract, or standard trolox, was added to 190 µL of ABTS radical solution in a 96-well microplate. The mixture was incubated at room temperature in the dark for 30 min. Then, absorbance readings were taken at 734 nm. Tests were carried out in triplicate.

The percent radical scavenging activity of extracts and compounds against ABTS radical were calculated according to the following: ABTS radical-scavenging activity (%) = $[(A_0-A_1)/A_0] \times 100$ where A_0 is the absorbance of the control (containing all reagents except the test extracts/compounds) and A_1 is the absorbance of the extracts/compounds. The extract (or standard) concentration resulting in a 50% inhibition (IC_{50}) was calculated by regression equations (by plotting the extract/standard solution concentration versus percentage of inhibition). Lower IC_{50} values indicate higher inhibitory potential of the tested extract (16).

The anti-lipoxygenase activity was evaluated as described by Phosrithong and Nuchtavorn (17) with slight modifications described by Yıldırım et al. (18). 10 µL of extract or standard indomethacin were added to 20 µL ethanol, 20 µL pure water, 25 µL of sodium borate buffer solution (0.1 M, pH 9) followed by an addition of 25 µL of type V soybean lipoxygenase solution in buffer (pH 9, 20.000 U/mL). The mixture was preincubated at 25 °C for 5 min. Then, 100 µL of 0.6 mM linoleic acid solution was added, mixed well and the change in absorbance at 234 nm was recorded for 6 min. Tests were carried out in triplicate. The inhibition percentage was calculated from the following equation: % inhibition= $[(A_{control}–A_{sample}) / A_{control}] \times 100$.

The extract (or standard) concentration resulting in a 50% inhibition (IC_{50}) was calculated by regression equations (by plotting the extract/standard solution concentration versus percentage of inhibition). Lower IC_{50} values indicate higher inhibitory potential of the tested extract (16).

Determination of the Total Phenolic and Flavonoid Contents

The total phenolic content of the extract was measured as described by Gao et al. (19) with slight modifications described by Yıldırım et al. (18) Ten µL of the extract in various concentrations was mixed with 20 µL of the Folin-Ciocalteu reagent, 200 µL of H_2O, and 100 µL of 15% Na_2CO_3, and the absorbance was measured at 765 nm after 2 h of incubation at room temperature. The total phenolic content was calculated on the basis of the calibration curve of standard gallic acid and expressed as mg gallic acid equivalent (GAE) per g dried extract.

Total flavonoid content was determined following a method by Zhang et al. (20) with slight modifications described by Yıldırım et al. (18). 25 µL extract in various concentrations was mixed with 125 µL of ultra-pure water and 7.5 µL of 5 % $NaNO_2$. After 6 min, 15 µL of 10% $AlCl_3 \cdot 6H_2O$ was added. After 5 min, 50 µL NaOH (1 M) was added and the solution completed with 250 µL of ultra-pure water. The absorbance was measured against the reagent blank at 510 nm. The total flavonoid content was calculated on the basis of the calibration curve of standard quercetin and expressed as mg quercetin equivalent (QE) per g dried extract.

Animals

A total of 24 female Sprague-Dawley rats (250-260 g; 4-5 months) were obtained from the Animal Centre (DEHAMER, Istanbul). The animals were housed in laboratory conditions and fed *ad libitum*. All protocols were carried out following the project submitted to the Marmara University Animal Care and Use Committee (Protocol number: 46.2020.mar). All animal experiments complied with the guidance of the Council of International Organization of Medical Sciences (WHO/UNESCO), NIH and PHS.

Animal Experimental Procedure

After 20 h of fasting, colitis was induced under anaesthesia, where an 8-cm soft cannula was inserted into the rectal hole and pushed forward. Subsequently, 5% (v/v) AA diluted in saline (pH 2.3) was administered in a volume of 1 mL (2). The control group was administered 1 mL saline intrarectally in lieu of AA. The rats were divided into two main groups: the control group and the AA-induced colitis group. The colitis group was further divided into three subgroups: the saline-treated (AA), the MoC extract-treated (AA+MoC), and SS-treated (AA+SS) groups, with each group consisting of 6 rats. Immediately after colitis induction and for the next 2 days, while saline was given to the control and AA groups, MoC extract (300 mg/kg) was given to the AA+MoC group and SS (100 mg/kg) was given to the AA+SS group. Thus, all treatments were given a total of 3 times. MoC extract and dissolved SS were administered to the rats using an oral gavage tube (p.o.) The dose of the MoC extract was selected based on a former study that demonstrated the protective effect of MoC on the Crohn's colitis model (10). However, SS, which is the standard drug for the treatment of ulcerative colitis, was used as a reference to evaluate the effectiveness of MoC (2). All treatments were given at around 11: 00 am. The rats were euthanized (Thiopenthal sodium, 50 mg/kg/i.p.) at the 72nd h of the colitis induction. The last 8 cm of the distal part of the colon was harvested. Colon tissues were taken for macroscopic and histological examinations, while the remaining parts were kept at -80°C until biochemical experiments were performed.

IL-1β, Malondialdehyde (MDA) and Glutathione (GSH) Levels and MPO Activity Measurement in Colonic Tissues

An enzyme-linked immunosorbent assay (ELISA) test was performed to determine IL-1β levels in the colonic samples following the manufacturer's instructions (EBIOSCIENCE, Thermo Fisher Scientific, MA, USA). MDA levels were defined for end products of membrane lipid oxidation by capturing the generation of thiobarbituric acid-sensitive substances as previously described by Buege and Aust (21) and the data were expressed as nmol MDA/g tissue. Cellular antioxidant GSH levels were determined using a modified Ellman procedure (22) and the data

were expressed as μmol GSH/g tissue. MPO activity assays were used in the evaluation of tissue neutrophil recruitment in line with Bradley et al. (23) and the data were expressed as U/g.

Immunoblotting

Frozen tissues were prepared in a 20 mM Tris-HCl buffer containing protease inhibitors. Following centrifugation, the pellets were incubated in the protease inhibitor solution (which includes DTT, glycerol, Tris-HCl, EDTA, and Triton X-100) for 1h. After determining the amount of protein in each of the colon tissues by the Lowry method (24), samples containing 100 mg of protein were prepared for SDS-PAGE separation. The prepared samples were loaded onto the gel and subsequently transferred to nitrocellulose membranes (at 90 V for 1 h). The transferred samples were incubated with primary antibodies [β-actin (sc-130657, 1: 200), bax (sc-20067, 1: 100), bcl-2 (sc-7382, 1: 200), casp-3 (sc-56053, 1: 200) and casp-9 (sc-56076, 1: 200] for 14 h, and then the membranes were incubated with rabbit monoclonal anti-goat IgG secondary antibodies (1: 1500) for an additional 1 h. All incubations with antibodies were carried out at +4 °C. A publicly available software was used for the densitometric analysis of the resulting membranes (Bio-Rad Molecular Analyst, www.totallab.com).

Morphological and Histopathological Analysis

Macroscopic scoring of the colonic damage was performed using the criteria outlined in Table 1, with a maximum score of 10, and photographed according to Karakoyun et al. (2). The colonic tissue samples were processed for light microscopic investigations. In short, the tissues were fixed in 10% formaldehyde, dehydrated in ethanol series with ascending alcohol concentrations, cleared in xylene, and embedded in paraffin blocks. The cut tissue sections (5 μm thick) were stained with hematoxylin and eosin (H&E) and photographed using a light microscope with its dedicated camera (Olympus BX51; Olympus DP72; Olympus, Tokyo, Japan). Semi-quantitative histological scoring was performed according to the method modified from the previous study (2) The relevant scoring criteria are stated in Table 2.

Table 1. Criteria of the macroscopic scoring of the colonic injury.

Score View
0 Normal view
1 Local hyperaemia, no ulcers
2 Non-hyperaemic ulceration and bowel wall thickening
3 Ulceration with inflammation at one region
4 Ulceration and inflammation in two or more regions
5 Colonic damage more than 1 cm
6-10 Damage is increased by 1 point for each cm
(Maximum score 10)

Table 2. Criteria of the microscopic scoring of the colonic injury.

Score	Appearance
0 None; 1 Mild; 2 Moderate; 3 Severe	Submucosal oedema
0 None; 1 Localized; 2 Moderate; 3 Severe	Damage/necrosis
0 None; 1 Mild; 2 Moderate; 3 Severe	Inflammatory cell infiltration
0 None; 1 Mild; 2 Moderate; 3 Severe	Haemorrhage
0 Absent; 1 Present	Perforation
(Maximum score 13)	

Statistical Analysis

GraphPad software (Prism 6.0; GraphPad Software, San Diego, CA, USA) was used for statistical analysis. The comparisons through all groups were made with analysis of one-way variance followed by Bonferroni multiple comparison post-hoc tests. All data were expressed as mean±SD, and $p<0.05$ was conceded to be statistically significant.

RESULTS

Extraction Yield, *In Vitro* Biological Activity, and Total Phenol and Flavonoid Content of MoC Extract

22.04 g dried MoC extract was obtained from 411.71 g fresh fruit (extraction yield: 5.35%). MoC extract displayed moderate DPPH radical scavenging activity with an IC_{50} of 222.70±12.16 μg/mL when compared to an IC_{50} value of 17.6±0.37 μg/mL of standard ascorbic acid ($p<0.05$). The extract showed moderate ABTS radical scavenging activity with an IC_{50} value of 349.30±3.47 μg/mL when compared to standard Trolox (IC_{50}=13.00±0.21 μg/mL; $p<0.05$). MoC extract with an IC_{50} value of 14.46±1.33 μg/mL showed potent anti-inflammatory activity compared to standard Indomethacin (IC_{50}=22.39±0.26 μg/mL; $p<0.05$). The total phenolic and total flavonoid contents of the extract were 26.16±2.01 mg GAE and 1.88±0.02 mg QE per g extract, respectively (Table 3).

IL-1β, MDA and GSH Levels, MPO Activity

The pro-inflammatory cytokine IL-1β levels of the saline-treated AA group were found to be significantly higher than those of the control group ($p<0.001$). The treatment of the rats with MoC and SS decreased IL-1β elevation ($p<0.01$-$p<0.001$, respectively; Figure 1a).

In comparison to the control group, while a significant elevation was observed in the MDA levels which indicate lipid peroxidation of the saline-treated AA group ($p<0.01$), this elevation significantly decreased in the MoC and SS treated colitis groups ($p<0.01$; Figure 1b). The cellular antioxidant molecule GSH levels were markedly diminished in the saline-treated AA group in comparison to the control group ($p<0.01$); however, a significant elevation of GSH levels was detected in the MoC and SS-treated colitis groups ($p<0.01$ and $p<0.01$,

Table 3. Anti-inflammatory, antioxidant activity and total compound content of MoC extract.

Assays	MoC*	Trolox	Ascorbic acid	Indomethacin
ABTS radical scavenging activity (IC_{50}, µg/mL)	349.30±3.47[b]	13.00±0.21[a]		
DPPH radical scavenging activity (IC_{50}, µg/mL)	222.70±12.16[b]		17.6±0.37[a]	
Anti-lipoxgenase activity (IC_{50}, µg/mL)	14.46±1.33[a]			22.39±0.26[b]
Total phenolic content (mg GAE/g extract)**	26.16±2.01			
Total flavonoid content (mg QE/g extract)***	1.88±0.02			

*MoC: Ethanol extract of *Momordica charantia* L. fruits
**Total phenolic content was expressed as gallic acid equivalent (GAE).
***Total flavonoid content was expressed as quercetin equivalent (QE).
****Each value in the table is represented as mean±SD (n=3). Different letter superscripts in the same line indicate significant differences (p<0.05).

respectively; Figure 1c). MPO activity, which indicates tissue neutrophil recruitment, was found to be significantly increased in the saline-treated AA group in comparison to the control group (p<0.001). The colonic MPO activity was decreased upon MoC and SS treatments in comparison to the saline-treated AA group (p<0.01 and p<0.001, respectively; Figure 1d). There was no statistical difference between MoC and SS treatments.

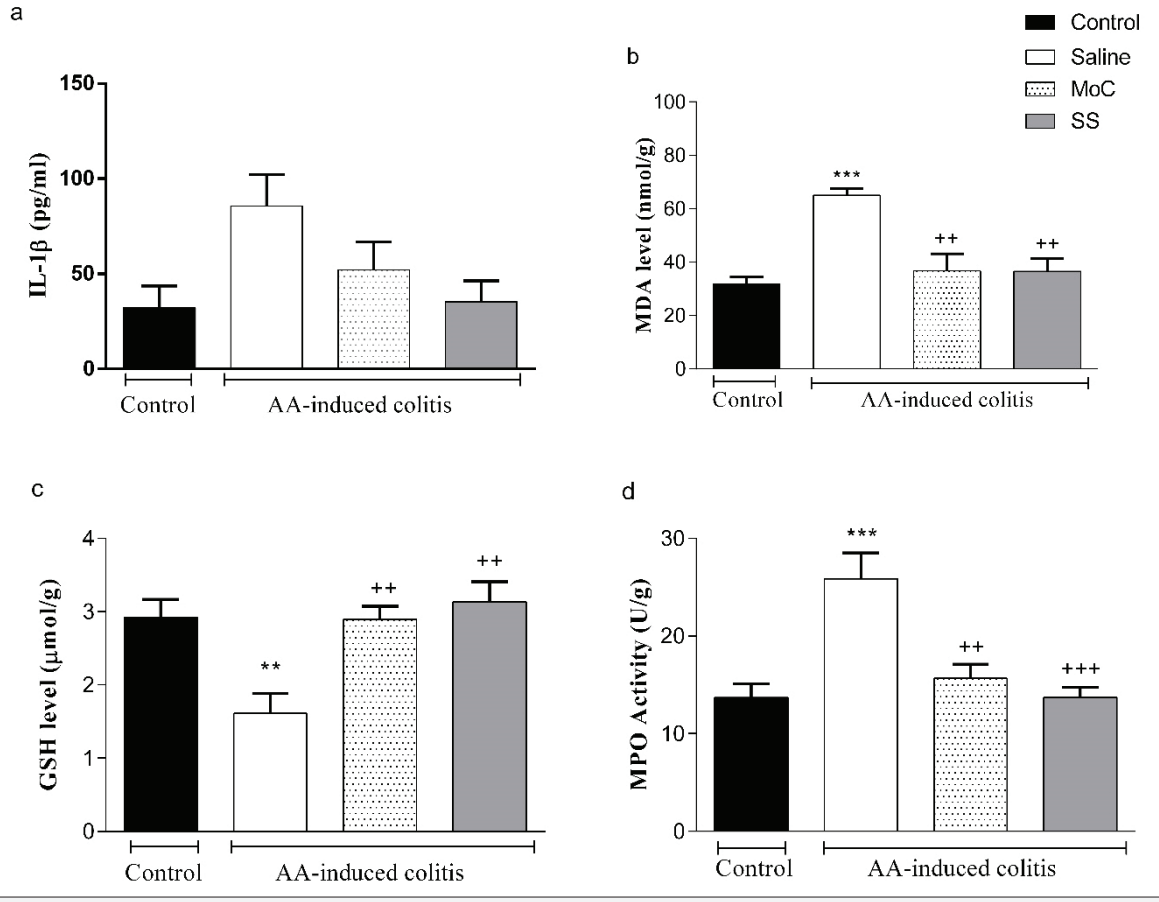

Figure 1. Tissue IL-1β (a), MDA (b), GSH levels (c), and MPO activity (d). $^{**}p<0.01$, and $^{***}p<0.001$ Comparisons according to the control group, $^{++}p<0.01$ and $^{+++}p<0.001$ Comparisons according to the saline-treated AA-induced colitis group. Control: Saline-treated control group, AA: Saline-treated AA-induced colitis group, MoC: *MoC* extract-treated AA-induced colitis group, SS: SS-treated AA-induced colitis group (for each group, n=6). Abbreviations: Interleukin 1-β (IL-1β); Malondialdehyde (MDA); Glutathione (GSH); Myeloperoxidase (MPO); Acetic acid (AA); Sulfasalazine (SS); *Momordica charantia* L. (MoC).

Immunoblotting

The representative immunoblotting membranes of the colon tissues were illustrated in Figure 2a. Changes in the bax/bcl-2 expression ratio and casp-9, casp-3 levels were used to determine the mitochondrial apoptosis evaluation for each group (*n*=4).

The bax/bcl-2 ratio was increased in the saline-treated AA-induced colitis group compared to the control group (p<0.001). The changes in the expression of bax/bcl-2 ratio in the AA-induced colitis group were restored upon MoC and SS treatments (p<0.01 and p<0.001, respectively; Figure 2b). Casp-9 and casp-3 levels were also significantly increased in the saline-treated AA-induced colitis group compared to the control group (p<0.05 and p<0.01, respectively). MoC and SS treatments decreased casp-9 and casp-3 levels compared to the saline-treated AA group (p<0.05; Figure 2c and 2d).

Morphological and Histopathological Analysis

At 72 h after colitis induction, macroscopic scoring was found to be significantly higher in the saline-treated AA group compared to the control group (p<0.001), while this scoring was significantly decreased in the MoC and SS-treated colitis groups (p<0.001; Figures 3a and c).

As a result of light microscopic examinations, colon tissues of the control group had histological layers with regular morphology and the total microscopic damage score was the lowest (mean 1.46; Figure 3b). In the saline-treated AA group, severe loss of epithelial lining, extensive submucosal oedema, massive inflammatory cell infiltration, and haemorrhage were observed with the highest total damage score (mean 12.4). MoC and SS-treated colitis groups showed similar histological appearance, such as more regular epithelial surfaces, decreased inflammatory cell infiltration, and submucosal oedema (Figure 4). Microscopic scoring was found to be markedly higher in the AA group compared to the control group (p<0.001), while this scoring was significantly decreased upon MoC and SS treatment (p<0.001). When compared to the control group, there were still high scores in the MoC and SS groups (p<0.01 and p<0.001, respectively), whereas the difference between these treatment groups was not significant (Figure 3b, Table 4).

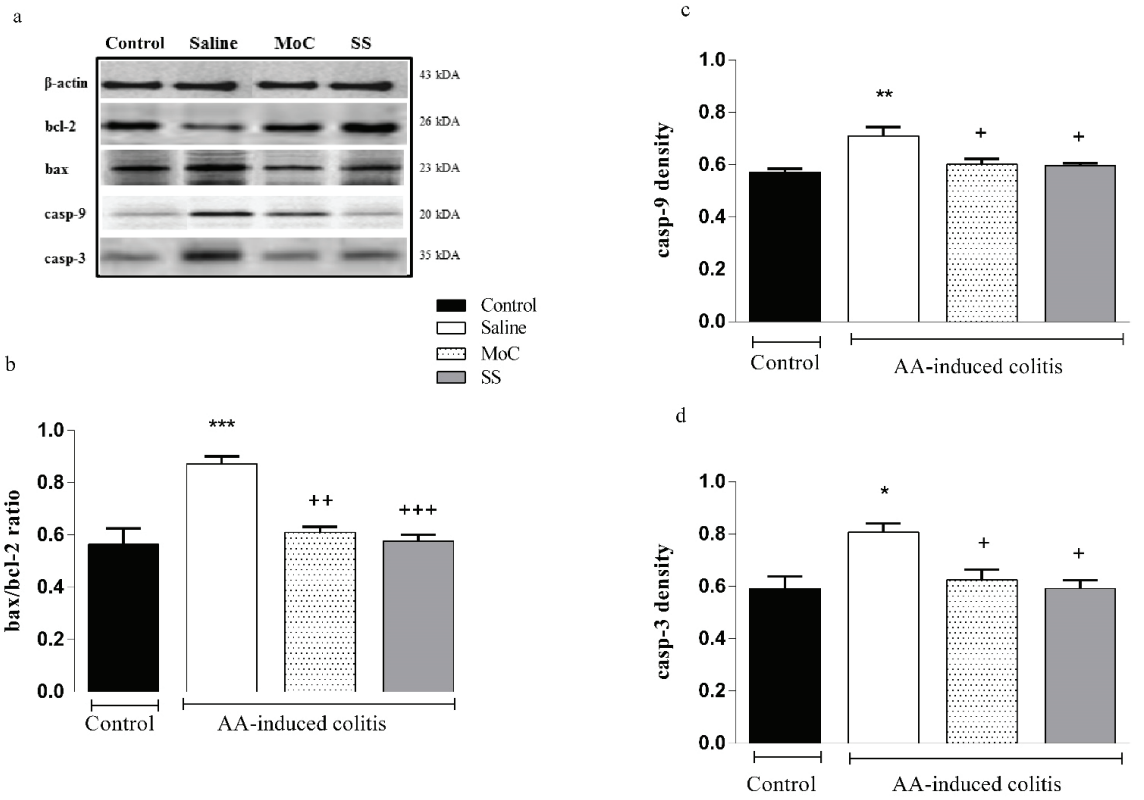

Figure 2. The representative images of membranes obtained from western blotting assays showing protein expressions of β-actin, bax, bcl 2, casp-9, and casp-3 (a), the expression levels of bax/bcl-2 ratio (b), casp-9 (c) and casp-3 (d) in colon.
All membranes (for each group, n=4) were normalized by using β-actin antibodies . *p<0.05, **p<0.01, and ***p<0.001 Comparisons according to control group, +p<0.05, ++p<0.01 and +++p<0.001 Comparisons according to saline treated AA-induced colitis group. Control: Saline-treated control group, AA: Saline-treated AA-induced colitis group, MoC: Momordica charantia extract-treated AA-induced colitis group, SS: SS-treated AA-induced colitis group (for each group, n=6). Abbreviations: Bcl-2-associated X protein (bax); B-cell lymphoma 2 (bcl-2); Caspase-9 (casp-9); Caspase-3 (casp-3); Acetic acid (AA); Sulfasalazine (SS); *Momordica charantia* L. (MoC).

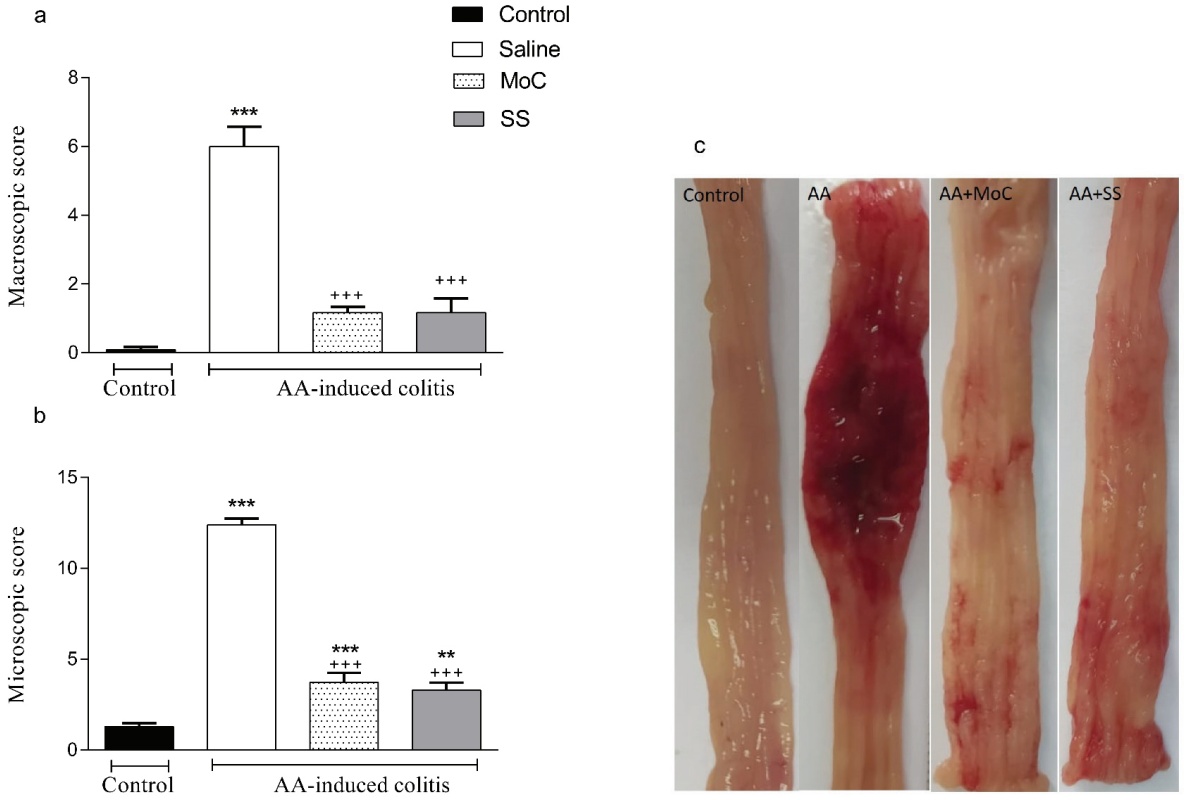

Figure 3. Macroscopic (a), microscopic scoring (b), and representative photographs of the colonic tissues (c). **p<0.01, and ***p<0.001 Comparisons according to control group, $^{+++}$p<0.001 Comparisons according to saline-treated AA-induced colitis group. Control: Saline-treated control group, AA: Saline-treated AA-induced colitis group, MoC: MoC extract-treated AA-induced colitis group, SS: SS-treated AA-induced colitis group (for each group, n=6). Abbreviations: Acetic acid (AA); Sulfasalazine (SS); *Momordica charantia* L. (MoC).

Table 4. Predominant histological grading of each sign of colonic damage in each experimental group of animals.

	Oedema	Damage/Necrosis	Inflammatory cell	Haemorrhage	Perforation	Total Score
Control	0.38	0.42	0.44	0.02	0.20	1.46
Saline+AA	3.00	2.70	3.00	2.90	0.80	12.40
MoC+AA	0.90	0.86	1.22	0.56	0.20	3.74
SS+AA	0.80	0.65	1.125	0.50	0.25	3.30

Abbreviations: Acetic acid (AA); Sulfasalazine (SS); *Momordica charantia* L. (MoC).

DISCUSSION

The results of the current study reveal that MoC extract ameliorates colonic damage, as assessed by decreased pro-inflammatory cytokine IL-1β levels, bax/bcl-2 ratios, caspase-3 and -9 levels, MPO activity, lipid peroxidation and increased GSH levels. Also, macroscopic and histopathologic evaluation suggests significant colonic healing.

In the present study *in vitro* DPPH and ABTS radical scavenging activity and lipoxygenase inhibitory activity, and *in vivo* MPO enzyme activity of MoC extract were investigated. The antioxidant and anti-lipoxygenic effects of MoC extract detected *in vitro* confirmed its in vivo antioxidant and anti-inflammatory activity. Previous studies have shown that MoC contained cucurbitane-type triterpenes as the main compounds (8, 9, 25-27). Furthermore, some of these triterpene compounds have been reported to have significant antioxidant and anti-inflammatory activities (27, 28). Therefore, these cucurbitane-type triterpenes together with other compounds in MoC may be responsible for the antioxidant and anti-inflammatory activity of MoC. In our *in vitro* analysis MoC exhibited a strong anti-inflammatory activity compared to standard Indomethacin, which is an important

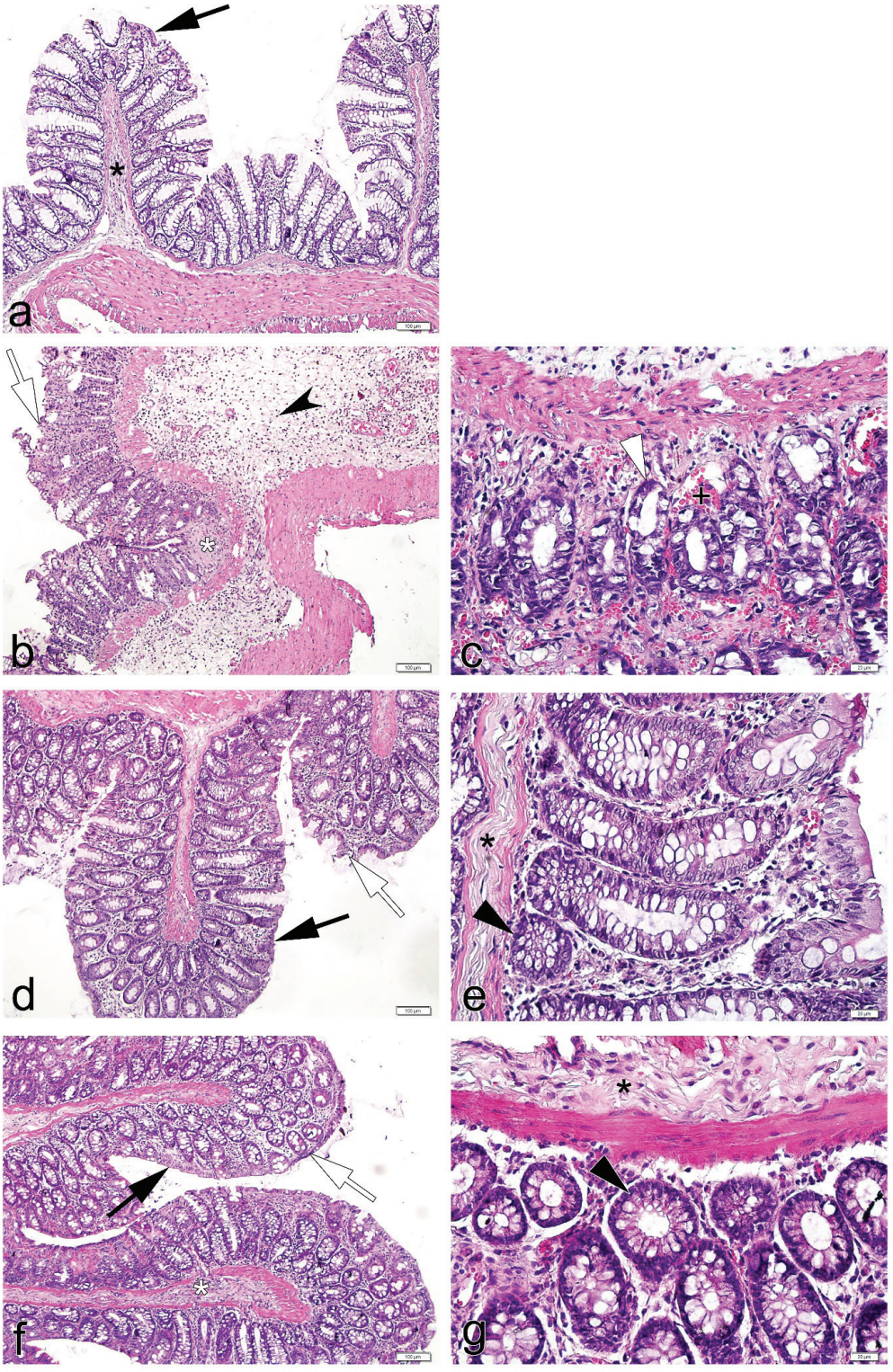

Figure 4. Representative light microscopic photographs of the colonic tissues from all experimental groups. (a) Saline-treated control group, (b-c) Saline-treated AA-induced colitis group (AA), (d-e) MoC extract-treated AA-induced colitis group (AA+MoC) group. (f-g) SS-treated AA-induced colitis group (AA+SS) group. Arrow: Normal colon mucosa with regular surface epithelium. Arrowhead: Regular Lieberkuhn crypts. Asterisk (*): Regular submucosa. Notched arrow: Inflammatory cell infiltration. Plus (+): Vascular dilatation/haemorrhage. White arrow: Colon mucosa with damaged epithelium. White arrowhead: Lieberkuhn crypts with the abnormal organization. H&E staining. Bar size a,b,d,f: 100 µm and c,e,g: 20 µm. Abbreviations: Acetic acid (AA); Sulfasalazine (SS); *Momordica charantia* L. (MoC).

reason for colonic healing. However, in the present study, the total phenolic and flavonoid contents of MoC were found to be low. The fact that MoC is rich in cucurbitane-type triterpene compounds overlaps with this result.

Here, we established an AA-induced colitis model because it is highly similar to human inflammatory bowel disease (IBD) in terms of pathophysiological, histopathologic, and inflammatory mediator profiles (29). AA-induced UC has been shown to initiate physical and chemical damage in colon tissue, causing inflammation and an increase in ROS levels and a subsequent decrease in GSH levels. Besides, neutrophils that infiltrate the damaged colon mucosa, which leads to oxidative stress, also increase inflammation (4, 30, 31). In the present study, impaired epithelial integrity, which was demonstrated macroscopically and microscopically, facilitated neutrophil infiltration from blood to colon tissue. Our *in vitro* biological activity results indicate that MoC extract has free radical scavenging and anti-inflammatory activities. Scavenging of free radicals and depletion of inflammation diminishes lipid peroxidation and restores cellular GSH stores. Decreased MDA levels and increased GSH levels observed upon MoC treatment in this study may be due to such activities of MoC. In addition, MoC therapy reduced MPO activity, which increased oxidative damage, and inflammation caused by AA increased neutrophil infiltration into the colon, mucosal and submucosal necrosis, oedema, vascular dilation, immense epithelial damage, and submucosal ulceration, which are main features of human colitis (29). Our macroscopic scoring showed that AA caused excessive damage to the colon tissue, but the MoC and SS treatments had significant ameliorative effects. Our macroscopic and microscopic results were in agreement with our biochemical results. Compared to the control group, there was still microscopic damage in the MoC and SS-treatment groups; however, these treatment groups were similar and the results correlated with macroscopic scoring. IL-1β proinflammatory cytokine is released from the colonic macrophage early in inflammation after AA administration and exacerbates mucosal inflammation (32, 33) and directly proportional to the severity of inflammation (34). Functional foods are beneficial by reducing pro-inflammatory cytokine expression in models of IBD (35). MoC reduced IL-1β levels in DSS-induced colitis and TNBS-induced colitis models (10, 15, 34).

In line with these studies, our result showed that colitis caused a significant increase in colonic IL-1β levels and that MoC and SS treatments were effective in reversing this trend. However, in those studies, the preparation methods of the plant sample, the treatment durations, treatment ways, and the chemical agents used for model induction were different from our study.

Colitis has previously been shown to cause increased apoptosis in colonic epithelial cells (36-38). Overproduction of ROS in cells can disrupt cellular parts, such as lipids and proteins, which can eventually cause cell death by apoptosis (39). Anti-apoptotic protein bcl-2 is localized on the mitochondrial membrane and inhibits apoptosis by stabilizing this membrane (40). However, bax inhibits the anti-apoptotic function of bcl-2 (41). Zhu et al. demonstrated the increased expression of bax/bcl-2, casp-3, and casp-9 in a UC rat model (38). Consistent with this report, our data showed that AA increased bax/bcl-2 ratio, casp-3, and casp-9 levels in the colon. In previous studies, MoC has been reported to reduce pro-apoptotic protein casp-3 and casp-9 levels of uterine tissue in ovariectomized rats (42). The results presented herein revealed that MoC extract attenuated AA-induced colonic cell apoptosis by decreasing casp-9 and casp-3 levels and bax/bcl-2 ratio, thereby improving the epithelial barrier integrity and colonic architecture.

CONCLUSION

Our *in vivo* and *in vitro* examinations proved that MoC extract had a protective role in AA-induced inflammation, apoptosis, and oxidative damage. MoC reduced the severity of colitis. Our results point out that MoC extract treatment appears to be as effective as SS treatment. According to our study results, we suggest that MoC has a protective role in AA-induced colitis.

Acknowledgement: We thank Deniz Mukaddes Turet for taking pictures of the colon and Seren Ede for helping arrange the references.

Ethics Committee Approval: This study was approved by the Marmara University Animal Experiments Local Ethics Committee (14.09.2020 / 45.2020.mar).

Informed Consent: Written consent was obtained from the participants.

Peer Review: Externally peer-reviewed.

Author contributions: Conception/Design of study- D.O., G.S.; Data Acquisition- D.O., A.S.; Data Analysis/Interpretation- D.O., A.S., A.A., K.T., O.T.C.K., I.S.; Drafting Manuscript- D.O., A.S., A.A., O.T.C.K.; Critical Revision of Manuscript- K.T, G.S.; All the authors reviewed the manuscript and after their respective inputs gave their final approval for submission/publication.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

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