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Original Article

Water Extraction of Plant (*Momordica Charantia)* Reduced Oxidative and Colonic Mucosal Inflammation in Colotic Male Balb/c Mice

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Abstract

Medicinal plants are the most easily accessible health resource for the community and frequently the people's preferred option. *Momordica charantia* (bitter gourd) is a member of the Cucurbitaceous plant used as a vegetable and in traditional medicine.

This study aimed to evaluate the effect of water extract of *Momordica charantia* leaves on male *Balb/c* mice with acute ulcerative colitis. 28 male mice were acclimatized and equally divided into four groups. The control group was given normal saline; the dextran sulphate sodium group (DSS) received 3% Dextran sulfate sodium in drinking water for seven days; the water extract of *Momordica charantia* + dextran sulphate sodium group (WEMC+DSS) received water extract of *Momordica Charantia* for seven days before dextran sulphate sodium (DSS) was administered; the water extract of *Momordica charantia* group (WEMC) received water extract of *Momordica charantia* (150mg/kg b.wt.) for 14 days. The mice were daily weighed and sacrificed on the 15th day of the experiment. Their colon and brain were collected for evaluation of disease activity indices (DAI) of colitis, and oxidative and inflammatory damage via biochemical and histological assessments. Results obtained showed no significant difference at ($p<0.05$) in DAI among the groups. The WEMC+DSS when compared with the DSS group had significantly higher catalase (272.9±54.8, 175.1+12.5, and 200.2±29.7), superoxide dismutase–SOD (347.7±37.5, 223.9±13.1, and 254.9±38.8), and glutathione peroxidase–GPx (28.6±2.20, 27.3±1.24, and 27.8±1.57) activities, and SOD/ (catalase+GPX) ratio (0.01±0.00, 0.003±0.00, and 0.01±0.002) and reduced hydrogen peroxide concentration (7.63±0.54, 4.33±1.28, and 5.71±0.75) in both colon and brain cytosolic fractions, respectively. Histological examination showed transmural inflammation in the DSS group as against mild mucosal inflammation in WEMC+DSS. This result shows that water extract of *Momordica charantia* has the potential to reduce colitis-induced oxidative damage and inflammation. Based on the results of this study, it can be concluded that the water extract of *Momordica charantia* leaves has the potential to mitigate oxidative damage and inflammation induced by ulcerative colitis in male Balb/c mice. Although there was no significant difference in disease activity indices among the groups, the group treated with the water extract of *Momordica charantia* before DSS administration showed significantly higher levels of antioxidant enzyme activities and lower levels of hydrogen peroxide concentration in both colon and brain cytosolic fractions compared to the DSS group.

Keywords: Ulcerative Colitis, *Momordica charantia*, Dextran Sulphate Sodium, oxidative damage, inflammation.

Introduction

Africa has vast biodiversity resources with estimates ranging between 40,000 and 45,000 species of plants with development potential with 5,000 species used for medicinal purposes [1]. Medicinal plants are the most easily accessible health resource for the community, and they are frequently the people's preferred option. A rising number of papers in the scientific literature are focused on analyzing the efficacy of African medicinal plants which are thought to play an essential role in the preservation of health and the introduction of new treatments. Furthermore, some plant species have already demonstrated their efficacy in the treatment of intestinal inflammation disorder [2].

Momordica charantia, sometimes known as (bitter gourd), is a member of the Cucurbitaceae plant that is primarily grown in Asia, Africa, and South America [3]. It has been long used as a vegetable

and for medicinal uses, including the treatment of inflammation, fever, and rheumatism, as well as anti-diabetic and anti-helmintic properties [4]. Several research has looked at the antioxidant, anti-inflammatory, anti-diabetic, anti-cancer, and metabolic regulating activities of entire edible components or extracts in vitro/or in vivo [5]. Bitter gourd's health benefits and biological activity have been studied. The presence of triterpenoids, saponins, proteins, and carotenoids is commonly linked to this [6].

Inflammatory bowel disease (IBD) is a life-long condition of chronic and relapsing inflammation of the gastrointestinal tract linked to an immune-mediated dysfunction that can last a lifetime [7]. It has two phenotypes: Crohn's disease, which can affect any portion of the intestine severely, and ulcerative colitis (UC), which entails destruction to the colon's beginning away from the anus [8]. IBD is widespread in affluent countries, with up to one in every 200 people in the Northern European region suffering from it [9]. IBD patients particularly those with UC, are at a higher risk of acquiring gastrointestinal and extra-intestinal carcinomas, with the chance of developing colitis-associated colorectal cancer (CACC) with the length and severity of inflammatory manifestation [10]. As a result, new approaches and alternatives to managing inflammation are required, and plant-derived chemicals have the potential to prevent, migrate, and alleviate chronic inflammation, as well as the risk of IBD and related disorders.

In past decades, dozens of different animal models of IBD have been developed. These models can be broadly divided into spontaneous colitis models, inducible colitis models, genetically modified models, and adoptive transfer models. Although these models do not represent the complexity of human disease, they are valuable and indispensable tools that provide a wide range of options for investigating the involvement of various factors in the pathogenesis of IBD and evaluating different therapeutic options [11]. Chemically induced murine models of intestinal inflammation are one of the most commonly used models because they are simple to induce, the onset, duration, and severity of inflammation are immediate and controllable [12].

Dextran sulfate sodium (DSS) is a sulfated polysaccharide with a highly variable molecular weight, ranging from 5kDa to up to 1400kDa. It was found that the molecular weight of DSS is a very important factor in the induction of colitis. Dextran sulfate sodium (DSS) is a well-established animal model of mucosal inflammation that has been used for 2 decades in the study of IBD pathogenesis and preclinical studies. The DSS-induced colitis model has some advantages when compared to other animal models of colitis. For example, an acute, chronic, or relapsing model can be produced easily by changing the concentration of administration of DSS (and cycle in rats and other strains of mice). This study evaluates the effect of plant extract on antioxidant activities, oxidative stress markers on disease activities induced ulcerative colitis.

Methodology

Plant Identification and Preparation of Extract

The leaves of *Momordica charantia* were locally sourced from a farm at Badagry, Lagos State, and were authenticated by the Botany Department at Lagos State University, Ojo, Lagos, state.

Preparation of Plant Materials

The plant was air dried at room temperature in the laboratory; it was examined daily for 7 days till the plant got completely dried after which it was ground to fine powder.

Soaking

Exactly 100 g and 70% of *Momordica charantia* (grounded) were soaked in 500 ml of distilled water and 300 ml of ethanol each were soaked for 72 hours. The mixture was concentrated after sieving with muslin and the filtrate obtained was filtered with number one Whatman filter paper. The residue was stored while the filtrate was put in the water bath, set at 50 °C to evaporate the solvent. The crude extract obtained from the 100 g was equivalent to a 25.51% yield.

Animal Procurement

Male albino mice (28) were obtained from Lagos State University College of Medicine (LASUCOM) and were maintained under standard laboratory conditions supplied daily with standard feed water except for the selected animals that were acclimatized (1 week). All the animal protocols conformed to the guiding principles of laboratory animal care (NIH Publication No. 85-93, revised 1985) [7] and were approved by the ad-hoc animal ethical committee (ref no. BCH-05/2007) of the Department of Biochemistry, Lagos State University, Ojo, Lagos, Nigeria. The animals were fed and kept in the Department of Biochemistry Lagos State University, Ojo, Animal House.

Induction of Ulcerative Colitis

Ulcerative colitis was induced in mice via the administration of DSS (3%) for one week through their drinking water; the control groups were given clean water instead of toxicant water.

The major determiner of a successful induction in this research was the presence of occult blood, and diarrhea, determined by carefully examining the anus of the mice induced with toxicant.

Body Weight Measurements

The body weights of the mice in each group were taken before the commencement of the oral administration using an electronic weighing scale and these weights taken were considered to be the initial body weight. The body weights of all groups were also recorded on the last day of oral administration, and these were the final body weights.

Animal Experimental Design

Four groups of animals were used for this study, each with seven animals. After the periods of experimentation, the mice were sacrificed then the colon and brain were harvested, homogenized in phosphate buffer, and centrifuged after being subjected to the biochemical evaluations.

Organ Preparation and Biochemical Assessment

Animal Dissection and Organ Weight Measurement

Animals of each group were sacrificed on the 15th day of experiment by cervical dislocation. A vertical midline incision was made with scissors from the neck to the pubis to open the peritoneum. The brain was quickly removed, washed in a phosphate buffered saline of pH 7.4, and then transferred by blunt forceps to a plain tube, homogenized in storage phosphate buffer, and kept frozen. The small intestines and colons were also removed, opened longitudinally, and

rinsed in cold phosphate-buffered saline of pH 7.4, weighed, and kept frozen to be used for biochemical assays.

Preparation of Cytosolic Fractions

The organs were homogenized using 0.1 M cold potassium phosphate buffer of pH 7.4 homogenized and centrifuged for 10 minutes at 4000 g, to pellet the nuclei. The supernatants were further centrifuged at 4000 g for 15 minutes to separate the cytosolic fractions, which were used for biochemical analysis.

Assessment of Biochemical Parameters

The following biochemical parameters were determined following the protocols indicated in parentheses: total protein concentration), catalase -CAT activity superoxide dismutase -SOD activity glutathione peroxidase (GPx) activity, reduced glutathione-GSH (Beutler, *et al.,*1963), glutathione-s-transferase-GST, and ion concentration–NO using Griess reaction protocol.

Total Protein Determination

The protein concentration of colon and brain cytosolic fractions was determined using a modified method of Biuret's test.

Determination of Catalase Activity

Exactly 2.95 mL of the 19 mM hydrogen peroxide solution was measured into a 1cm quartz cuvette using graded pipettes while sample addition followed immediately. The cuvette was covered and rapidly but gently mixed. Change in absorbance was recorded every thirty seconds for at least two minutes. Catalase activity was calculated using the following formula:

$$\text{Catalase activity} = (\text{Change in absorbance(min)} \times \text{Total volume of assay mixture}) / (\text{extinction coefficient} \times \text{volume of sample}) \quad (1)$$

Assessment of Lipid Peroxidation

Lipid peroxidation (LPO) was assessed through measurement of the peroxidation product (malondialdehyde) present in the samples. 0.5% TBA was diluted in 20% TCA (1:1). An aliquot of 0.5 ml each of post mitochondria fractions of the colon and brain and 1.5 ml of the mixture TBA+TCA were added to glass tubes and incubated in a water bath at 95 °C for 25 minutes to develop a colored complex. The tubes were cooled on ice to stop the reaction and centrifuged at 3000 rpm for 10 minutes the supernatant was collected, and absorbance was measured at 532nm and 600nm. The absorbance value of 600 nm was deducted from the values at 532nm and MDA content for each sample was calculated following Beer-Lambert law and using an extinction coefficient (Ɛ) =155 mM. results were expressed as nanomoles MDA produced/mg protein.

$$\text{LPO concentration} = (\text{Change in absorbance (min)} \times \text{Total volume of assay mixture}) / (\text{Extinction coefficient} \times \text{volume of sample used}) \quad (2)$$

Estimation of Reduced Glutathione Level

Aliquots of each sample were deprotonated by mixing of equal amount of both sample and Sulphosalicyclic acid in sample bottles. The precipitate was vortexed and centrifuged at 4000 g for five minutes. The supernatant was picked and 0.5 ml of it was mixed with 1.5 ml of DTNB while absorbance was read at 412

nm. Stock solution of reduced glutathione was serially diluted in 0.1 M phosphate buffer and mixed with DTNB (as of sample) and was used to generate values for a standard graph from which glutathione concentrations were extrapolated.

Determination of Superoxide Dismutase Activity

Exactly 100 µl of the sample was added to a cuvette containing 1.25 ml of 0.05 M carbonate buffer (pH 10.2) and 150 µl of epinephrine and gently mixed by inverting the cuvette. Change in absorbances was recorded at thirty-second intervals for three minutes at 480 nm wavelength. Distilled water was used instead of a sample to obtain a blank reading.

SOD activity = (Change in absorbance(min)×Total volume of assay mixture)/(Extinction coefficient ×volume of sample used) (3)

Determination of Hydrogen Peroxide Concentration

Stock hydrogen peroxide (30%) was diluted in distilled water, following which 1.9 ml of FOXI reagent was added to 100 µl of the serially diluted stock H_2O_2. The mixture was vortexed and incubated at room temperature for 30 minutes after which the absorbance was recorded at 560 nm wavelength against reagent blank. Some steps for standard were followed for samples, with 100µl of sample replacing the standard, but in addition, the whole mixtures were centrifuged at 3000 g for 5 minutes before incubation at room temperature. From the standard curve, the concentration of H_2O_2 in the samples was determined.

Estimation of Glutathione-S-Transferase Activity

20 Mm CDNB (100 µl) was put in a glass cuvette to which 20 µl of 0.1 M reduced glutathione, 1.86 ml of 0.1 M phosphate buffer (pH6.5), and 20 µl of distilled water was added. Samples were used in place of distilled water. Change in absorbance was recorded at thirty seconds interval for three minutes for samples and blank at 340 nm.
GST activity was calculated as:

GST activity = (Change in absorbance (min) × Total volume of assay mixture ×dilution factor)/(Extinction coefficient of adrenochrome ×volume of sample used) (4)

Where, Ɛ = extinction coefficient = 9.6 mM

Specific GST activity= GST activity/ mg protein

Assay for Glutathione Peroxidase (GPx) Activity

Measurement of the activity of glutathione peroxidase (GPx) was based on the modified method of Rotruck and colleagues (1973). Exactly 0.1 ml of NaN_3, 0.2 ml of GSH, 0.1 ml of H_2O_2, and 0.5 ml of sample were added in that order 0.5 ml of phosphate buffer in a test tube. The whole mixture was incubated in a water bath at 37 ºC for three minutes and 0.5 ml of TCA was thereafter added. The mixture was centrifuged for five minutes at 3000 g. the supernatant and removed. 2 ml of K_2HPO_4 and 1 ml of DNTB were added to 1 ml of supernatant and the absorbance was recorded at 412 nm against a reagent blank (in which distilled water replaced the sample)

Specific activity of GPx =GSH consumed / mg protein=µg GSH/mg protein (5)

Determination of Nitrite Ion Concentration (NO^{2-})

Griess and serially diluted standards as well as samples were mixed in separate test tubes, and incubated in the dark for twenty minutes at room temperature and the absorbance reading was taken at 546 nm wavelength. Nitrite concentrations were extrapolated from the sodium nitrite standard curve.

In Vitro Assessment of Momordica Charantia

Determination of Total Flavonoid Content

The TFC was measured following a spectrophotometric method [13]. 1 ml of the extract solution in different concentrations (25 µg/ml-500 µg/ml) was put into seven different test tubes using a micropipette, 0.3 ml of 5% sodium nitrite solution (5 g dissolved in 100ml of distilled water) was added to each test tube. After 5 minutes, 0.3 ml of 10% Aluminum chloride (10 g dissolved in 100 ml of methanol) was added. After 1 minute, 2 ml of 1.0 M sodium hydroxide was added, and 2.4 ml of distilled water was added. The mixture in each test tube was mixed well. The absorbance of the reaction mixture was read at 510 nm. A reagent blank was prepared using distilled water instead of the plant extract. The amount of phenolic compound in the extract was determined from the standard curve produced with varying concentrations (25 µg/ml-500 µg/ml) of Gallic acid. The total phenolic content of the plant was expressed as g Gallic acid equivalent (GAE)/100 g dry weight. All samples were analyzed in duplicate and averaged.

Estimation of Total Polyphenols

The total phenolic content in the plant extract of *Momordica charantia* was determined spectrophotometrically using the method by [14] with Folin Ciocalteau reagent. 10% Folin Ciocalteau reagent was prepared by adding Folin Ciocalteau reagent (10 ml) in (90 ml) of distilled water. 7.5% Na_2CO_3 was prepared by dissolving Na_2CO_3 in water (100 ml). The stock solution was prepared by dissolving 5 mg of each extract in 10 ml of the parent solvents. An aliquot of the extract (0.5 ml) was prepared into seven different test tubes in different concentrations (25 µg/ml-500 µg/ml), 2.5 ml of 10% Folin Ciocalteau reagent, and 2 ml of $NaCO_3$ (75% w/v) was added. The resulting mixture was vortexed for 15 seconds and incubated at 40ºC for 30 minutes for color development. The absorbance of the sample was measured at 765 nm. A reagent blank was prepared using distilled water instead of the plant extract. Total phenolic content was expressed as g Gallic acid equivalent (GAE)/100 g dry weight. The experiment was conducted in duplicate and the results are reported as mean ±SD values.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 9.0 statistical package (GraphPad Software, USA). The data were analyzed by one- or two-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. All values were expressed as mean ± standard error of the mean (mean ± SEM), and all results were considered to be statistically significant at an α-level of $P < 0.05$. Superscripts a, b, c, and d indicate groups that were significantly different from the control group, DSS group, WEMC group, and WEMC + DSS group.

Results

Graphical Representation of Biological Assay

To determine whether water extract of *Momordica charantia* could lead to a reduction in body mass within a short period of administration, changes in body weight of the mice in all the groups were determined and the result of Figure 1 shows that the control group gained more weight than DSS group when compared with WEMC and WEMC+DSS groups, but the differences in body weight gain were not statistically significant ($p < 0.05$).

Figure 2 A shows the % relative change in brain weight of the mice where there was no significant difference among the groups at $p < 0.05$, Figure 2B indicates the relative colon weight of the male mice where DSS was significantly different from WEMC + DSS and no significant difference at other groups at $p<0.005$, and Figure 2C demonstrates the colon length of the male mice where control is significantly different from DSS and, control is significantly from WEMC+DSS and no significant difference among other groups at $p<0.005$.

Figure 3A shows the specific reduced Glutathione activity of the colon and brain of male mice while there was no significant difference among the groups at $p<0.05$, Figure 3B demonstrates specific catalase activity of colon and brain weight of male mice with no significant difference among the groups at $p<0.05$, and Figure 3C presents the specific Glutathione-S-transferase activity of the colon and brain of male mice where the control and the DSS group of the colon were significantly different from the WEMC of the brain. DSS of the colon was significantly different from WEMC + DSS of the brain. WEMC of the colon was significantly different from the WEMC, WEMC + DSS of the brain. WEMC+DSS of the colon was significantly different from WEMC of the brain. Control and DSS of the brain were significantly different from WEMC of the brain and no significant difference among other groups at $p<0.005$. α indicates that the control group of the colon is significantly lower than the WEMC of the brain, θ demonstrates that the control group of the colon is significantly lower than the WEMC of the brain, ϕ shows that the control group of the colon is significantly lower than WEMC+DSS of the brain, ε illustrates that the control group of the colon is significantly lower than WEMC+DSS of the brain, λ indicate that the control group of the colon is significantly lower than WEMC of the brain, and e and f indicate that the control and DSS group of the brain is significantly lower than WEMC of the brain.

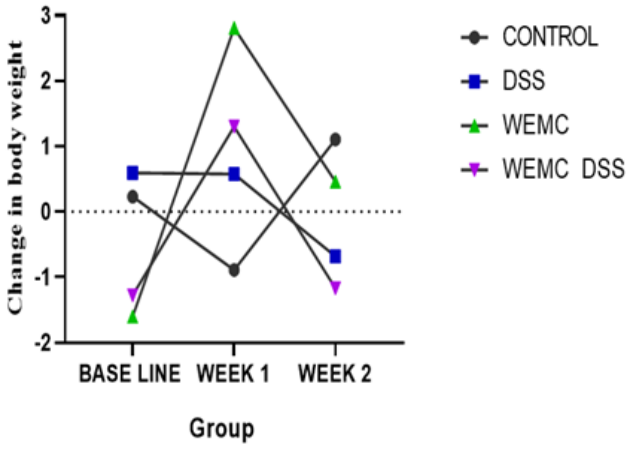

Figure 1 The relative change in mice body weight

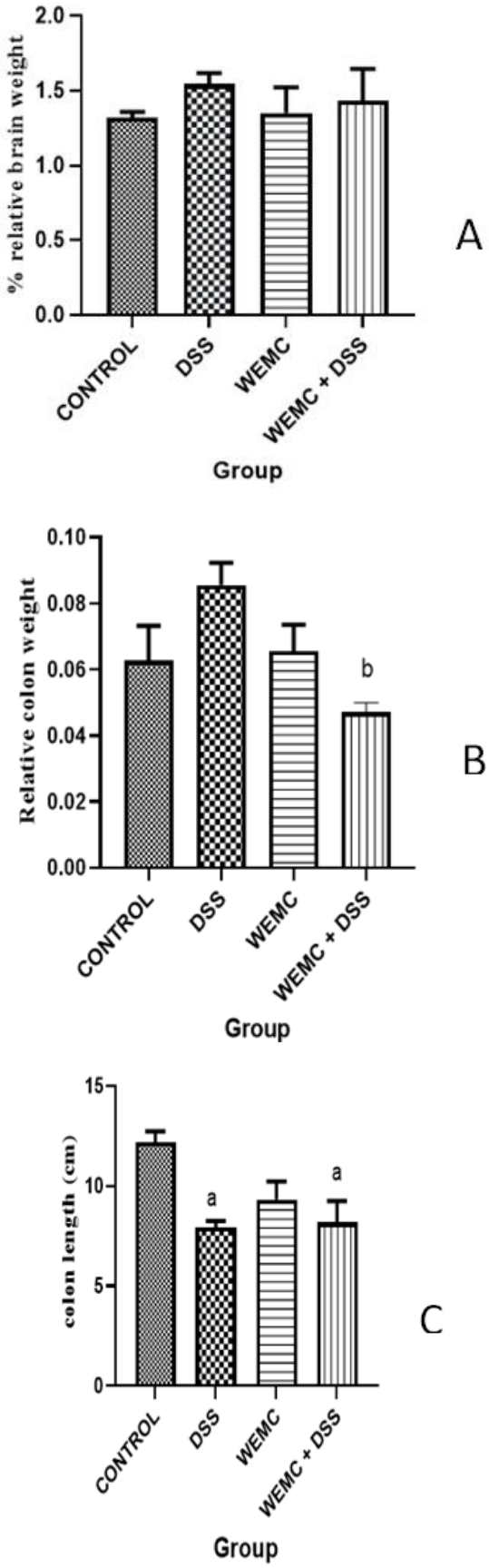

Figure 2 The percentage relative change in brain weight (A), relative colon weight of the mice (B) and the colon length of the male mice (C)

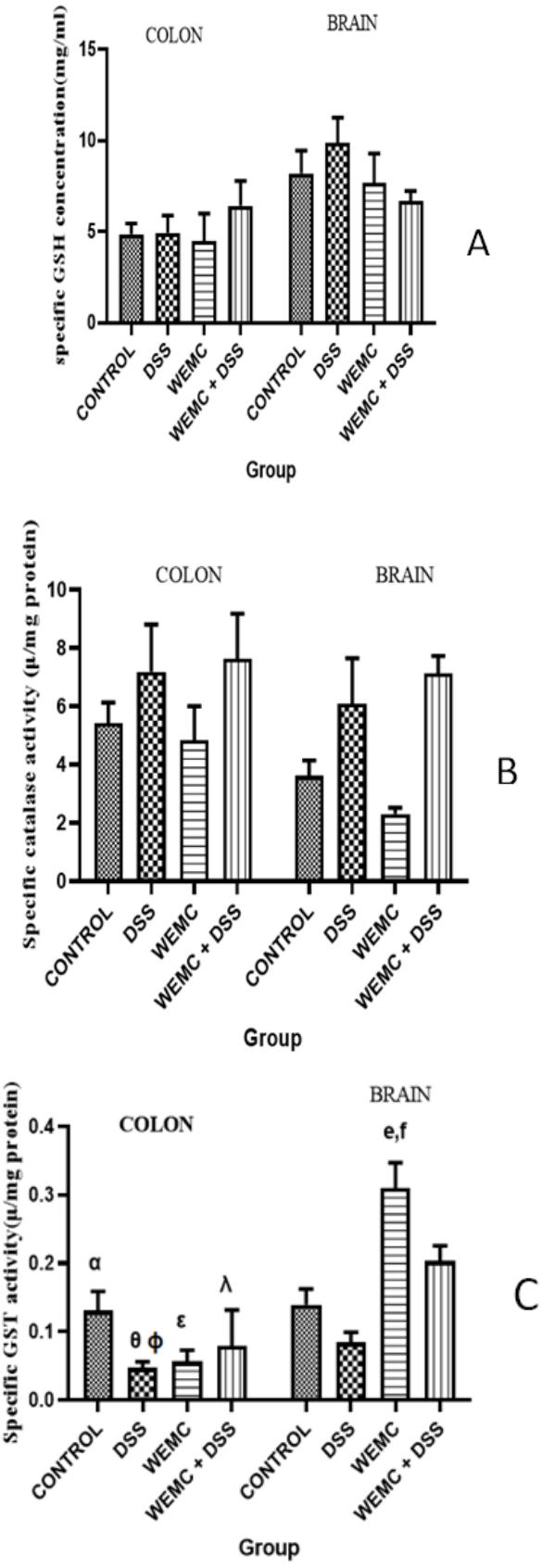

Figure 3 The specific reduced Glutathione activity of colon and brain of male mice (A), specific catalase activity of colon and brain weight of male mice (B) and the specific Glutathione-S-transferase activity of the colon and brain of male mice (C)

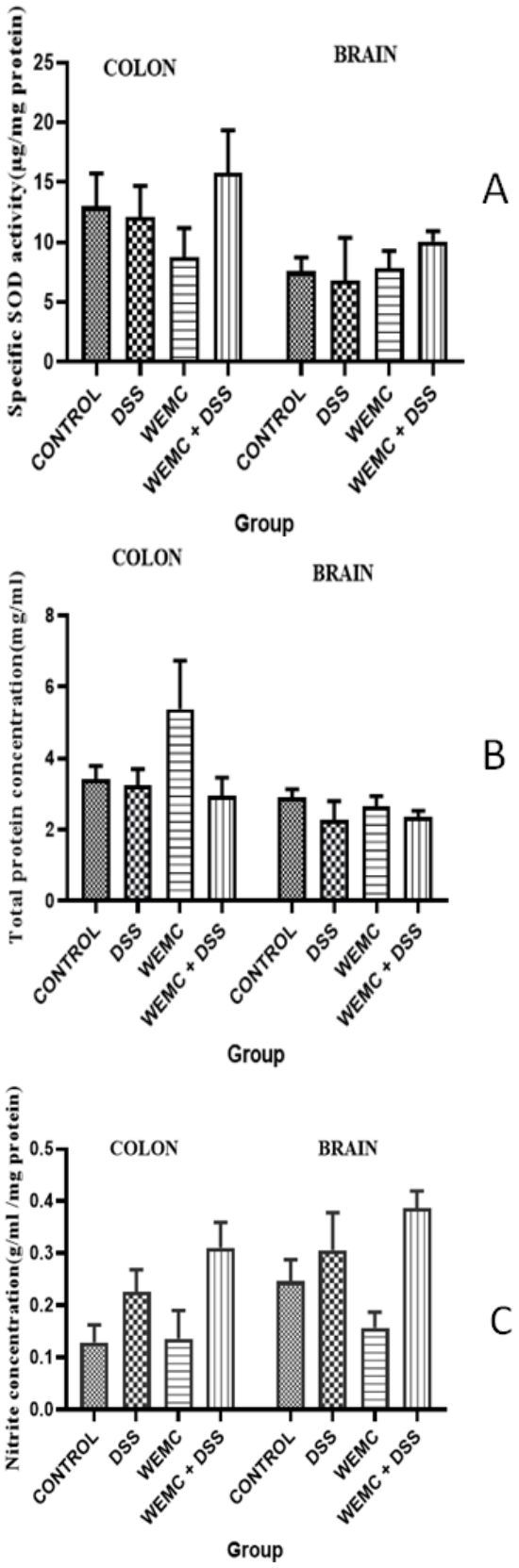

Figure 4 The superoxide dismutase activity of colon and brain of male mice (A), the total protein concentration of colon and brain of male mice (B) and the Nitrite oxide activity of colon and brain of male mice (C)

Figure 4A shows superoxide dismutase activity of the colon and brain of male mice with no significant difference among the group at p<0.05, Figure 4B the total protein concentration of colon and brain of male mice with no significant difference among the groups at p<0.05, and Figure 4C demonstrates the nitrite oxide activity of colon and brain of male mice where there was no significant difference among the groups at p<0.05.

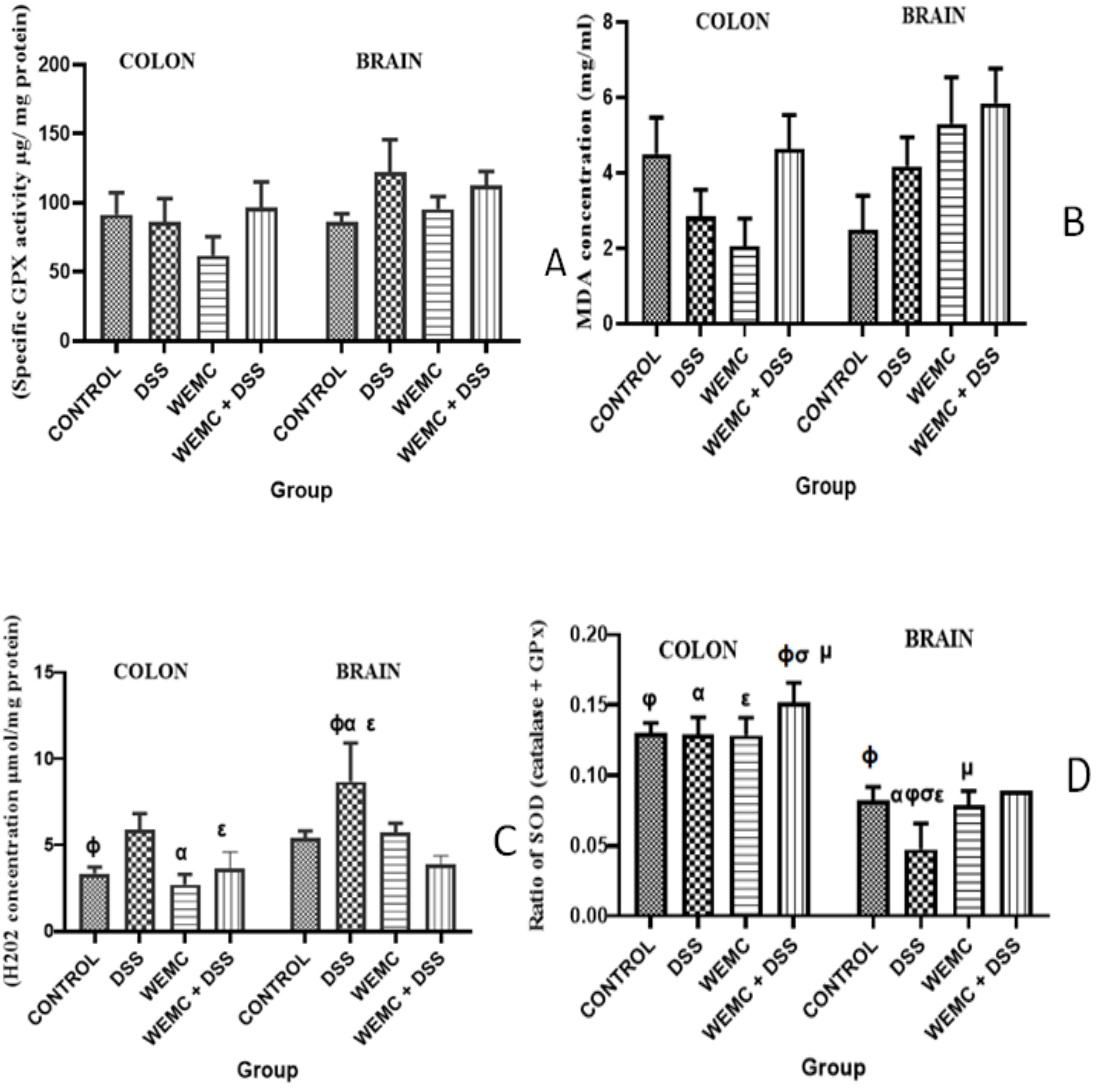

Figure 5 The specific Glutathione peroxidase activity of the colon and brain of male mice (A), malondialdehyde concentration of colon and brain of male mice (B), and hydrogen peroxide concentration of colon and brain of male mice (C)

Figure 5A shows the specific Glutathione peroxidase activity of the colon and brain of male mice with no significant difference among the groups at p <0.05, Figure 5B demonstrates malondialdehyde concentration of the colon and brain of male mice with no significant difference among the groups at p<0.05 and Figure 5C shows the hydrogen peroxide concentration of the colon and brain of male mice where the control group of the colon was significantly different from the DSS of the brain, WEMC of the colon was also

significantly different from the DSS of the brain. WEMC+DSS of the colon was significantly different from DSS of the brain and no significant difference among the groups at $p<0.05$.

ϕ indicates that the control group of the colon is significantly lower than the DSS of the brain, α indicates that the WEMC group of the colon is significantly lower than the DSS of the brain, and ε shows that the WEMC group of the colon is significantly lower than the DSS of the brain.

Figure 5D shows the ratio of SOD (Catalase + GPx) activity of the colon and brain of male mice where the control group of the colon was significantly different from the DSS of the brain. DSS of the colon was significantly different from the DSS of the brain, and the WEMC of the colon was significantly different from the DSS of the brain. WEMC + DSS of the colon was significantly different from control, DSS, and WEMC of the brain, and no significant difference among other groups at $p<0.05$.

φ indicates that the control of the colon is significantly higher than the DSS of the brain, α indicates that the DSS of the colon is significantly higher than the DSS of the brain, and ε indicates that the WEMC of the colon is significantly higher than the DSS of the brain.

ϕσ indicates that the WEMC+DSS of the colon is significantly higher than the DSS of the brain and μ shows that the WEMC+DSS of the colon is significantly higher than the WEMC of the brain.

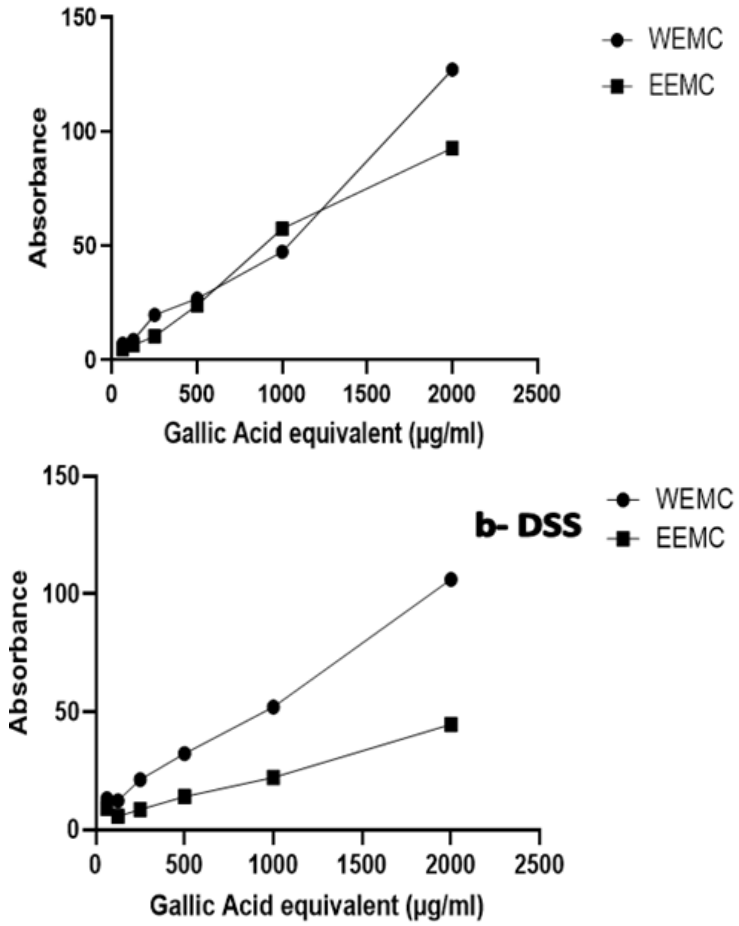

Figure 6 The flavonoid properties of EEMC and WEMC of *Momordica charantia,* the flavonoid properties of EEMC and WEMC of *Momordica charantia (A),* and the polyphenol properties of EEMC and WEMC of *Momordica charantia (B)*

Figure 6A demonstrates the flavonoid properties of EEMC and WEMC of *Momordica charantia* with no significant difference between the two groups at $p<0.05$ and Figure 6B illustrates the polyphenol properties of EEMC and WEMC of *Momordica charantia* with no significant difference between the two groups at $p<0.05$.

Histological Representation

Histological Assessment of Colon and Brain Sections

Figures 7 and 8 demonstrate photomicrographs of histological assessment of formalin-fixed paraffin-embedded colon and brain sections of selected mice from the four experimental groups.

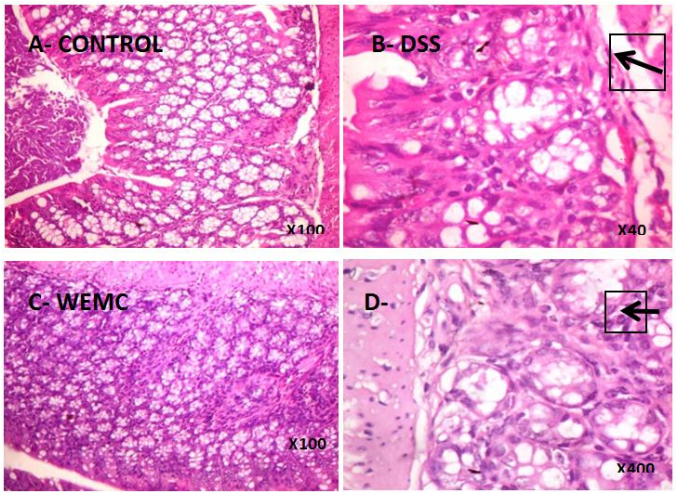

Figure 7 Photomicrographs of sections of the colon of (a) untreated male mice showing normal colon architecture; (b) male mice treated with DSS showing transmural inflammation which is prominent in the submucosa; (c) male mice treated with *Momordica charantia* WEMC showing normal colon architecture; and (d) male mice treated with *Momordica charantia* WEMC+DSS showing mild mucosal inflammation

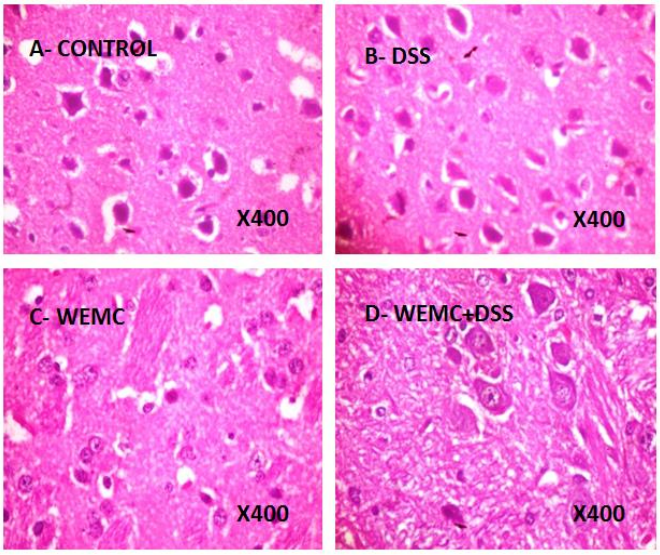

Figure 8 Photomicrographs of sections of brain of (a) untreated male mice –Control, (b) male mice treated with DSS, (c) male mice treated with *Momordica charantia* WEMC, and (d) male mice treated with *Momordica charantia* WEMC+DSS

All plates show normal brain architecture with no significant lesion (Magnification: x 400).

Discussion

The results of the present study demonstrated that treatment of DSS-induced colitis mice with *Momordica charantia* markedly decreased their colon damage. Moreover, parameters indicating the presence of oxidative injury were inhibited by *Momordica charantia* suggesting that *Momordica charantia* may have a potent anti-inflammatory effect on the inflamed colon and that they may also exert an adverse effect when its concentration is high in the body leading body weight loss [15].

Percentage change in the body weight of an animal can be used to assess the effectiveness of a particular therapy. Significant weight reduction may be associated with poor feed consumption among other causes [16]. No significant change in body weight was observed among the four groups of mice used in this study. *Momordica charantia* plant extracts caused a slight decrease in body weight compared with the control (which received only the control). Although studies have shown that MC extract can capably prevent excessive gain in body mass possibly due to the presence of some essential vitamins which are vital for healthy growth and development.

The colon length in this study was increased with the water extract of *Momordica charantia* compared to the DSS group which was shortened due to inflammation this is as a result of the high potency of the crude extracts. This extract can be used as management therapy for DSS.

Protective antioxidant enzymes such as SOD and CAT are the first line of defense against reactive oxygen species-ROS. Many normal biochemical processes that occur in eukaryotes involve the generation of controlled amounts of reactive oxygen and nitrogen species most often to serve as a self-defense mechanism against antigenic substances. These reactive species are normally removed from the system by specialized antioxidant defensive mechanisms which work in a concerted manner to prevent oxidative damage to cells and tissues [16]. However, when the levels of these radicals exceed the capacity of antioxidant molecules, oxidative stress results. The changes in the equilibrium between the formation of hydrogen peroxide by superoxide dismutation and its decomposition by the GPx and CAT in the organs are represented by the Ratio (R) = SOD/(CAT+GPx) which is the ratio of SOD-specific activity to the sum of CAT and GPx specific activities. A ratio less than one (1) is an indication of high antioxidant status, the ratio of exactly 1 indicates an equilibrium (*i.e*. a balance between the rate of ROS production and their breakdown), while a ratio above 1 indicates excessive ROS production with depleted levels or activities of antioxidant enzymes. The significant increase in SOD activity in the WEMC + DSS group for both colon and brain means that high levels of superoxide anions were generated in that group. To determine the antioxidant state of an organism, the ratio of SOD/(CAT+GPx) was determined in the organs. Although SOD/(CAT+GPx) was significantly higher in the group administered with the water extract of *Momordica charantia* leaf extract than in other groups, the ratio was not up to 1 which means none of the agents administered elicited a significant effect during the period of the experiment. However, oxidative stress might likely occur over prolonged administration of high doses of MC leaf extract which may lead to damage.

In a study, it was shown that an increase in colon oxidative stress can be

effectively counteracted by NO via its rapid inactivation of superoxide [17]. Since nitrite ion concentration is both beneficial and cytotoxic, one cannot conclusively say that the high levels observed in the WEMC + DSS groups were beneficial or not until a clear distinction is made between the endothelial and inducible forms of NO^{2-}. However, the significantly raised values compared to DSS should caution one concerning the dosage to use when administering the extracts for chronic studies [18]. The reduced level of H_2O_2 observed in the WEMC + DSS group compared to DSS, and control is a result of Superoxide anion (O^{2-}) produced by Superoxide dismutase and their conversion by CAT or GPx to less reactive agents such as H_2O_2 or their complete breakdown to oxygen and water.

The findings of this study regarding the activities of catalase (CAT), glutathione peroxidase (GPx), and glutathione (GSH) concentration in the colon and brain of mice treated with Momordica charantia extract and DSS (WEM + DSS) are consistent with previous research. Elevated activities of CAT in both colon and brain tissues indicate an increase in antioxidant defense mechanisms, which may help mitigate oxidative damage. Similarly, the improved activity of GPx, coupled with the observed increase in GSH concentration, suggests enhanced antioxidant capacity, as GSH serves as a co-substrate for GPx. This aligns with previous studies demonstrating the effectiveness of Momordica charantia in improving antioxidant enzyme activities and GSH levels, contributing to overall antioxidant defense against oxidative stress. The correlation between increased CAT and GPx activities and the corresponding changes in GSH concentration further supports the notion that Momordica charantia exerts its protective effects by enhancing antioxidant pathways in both colon and brain tissues [19]. Overall, these findings strengthen the evidence for the potential therapeutic benefits of Momordica charantia in reducing oxidative damage and inflammation associated with conditions such as ulcerative colitis.

The histological findings in this study are consistent with previous research work, which also demonstrated that mice treated with DSS alone exhibited transmural inflammation, particularly prominent in the submucosa, while the control group maintained normal colon architecture [20]. However, in contrast to the DSS group, mice treated with Momordica charantia water extract and DSS (WEMC+DSS) showed only mild mucosal inflammation, indicating a potential protective effect of the Momordica charantia extract against colitis-induced inflammation. Furthermore, the examination of brain tissue revealed normal architecture with no significant lesions, suggesting that the treatment did not adversely affect brain morphology. This comparison reinforces the potential therapeutic benefit of Momordica charantia in ameliorating colitis-associated inflammation and supports its consideration as a natural remedy for ulcerative colitis.

Different factors such as the dosage, and the treatment duration might have contributed to the difference in activity and the concentration of the antioxidant enzymes. The higher polyphenol and flavonoids observed in the water extract compared to ethanol extract possibly means that water extract contains higher antioxidant capacity compared to ethanol extract which also supports a previous study that the solvent used in extraction determines the type of flavonoids and polyphenol that will be obtained.

Conclusion

This result has shown that administration of a low dosage of the crude extracts (150 mg/kg) which is not potent enough to reduce the symptoms and disease activities in disease-induced colitis within one week, the dosage needs to be slightly increased in the further studies to increase the potency of the crude extracts. Hence, a slightly high dose may be recommended to ameliorate the disease.

Conflict of Interest

The authors declared that they have no affiliations with or involvement with any organization or entity with any financial interest such as honoraria, educational grants, participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or any other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Consent for Publication

Consent given.

Availability of Data and Materials

All supporting data are available on request.

Authors Contributions

All co-authors participated in all stages of this study while preparing the final version. All authors read and approved the final manuscript.

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Ethical Consent

The authors declared that Ethics Committee approval was gotten for this study as animals were sacrificed or used in the study. The work also contains plants study with data collection from online resources freely available in the public domain that does not collect or store identifiable data. All related laws, rules, and regulations necessary for the study execution have been followed.

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References

1. Halilu EM. Cultivation and conservation of african medicinal plants for pharmaceutical research and socio-economic development. inmedicinal Plants 2022 Nov 2. *IntechOpen*. [Crossref], [Google Scholar], [Publisher]
2. Aware CB, Patil DN, Suryawanshi SS, Mali PR, Rane MR, Gurav RG, Jadhav JP. Natural bioactive products as promising therapeutics: A review of natural product-based drug development. *South African Journal of Botany.* 2022 Dec 1;151:512-28. [Crossref], [Google Scholar], [Publisher]
3. Palamthodi S, Lele SS. Nutraceutical applications of gourd family vegetables: Benincasa hispida, Lagenaria siceraria and Momordica charantia. *Biomedicine & Preventive Nutriti*on. 2014 Jan 1;4(1):15-21. [Crossref], [Google Scholar], [Publisher]
4. Lee SY, Wong WF, Dong J, Cheng KK. Momordica charantia suppresses inflammation and glycolysis in lipopolysaccharide-activated RAW264. 7 macrophages. *Molecules*. 2020 Aug 20;25(17):3783. [Crossref], [Google Scholar], [Publisher]
5. Dandawate PR, Subramaniam D, Padhye SB, Anant S. Bitter melon: a panacea for inflammation and cancer. *Chinese Journal of Natural Medicines*. 2016 Feb 1;14(2):81-100. [Crossref], [Google Scholar], [Publisher]
6. Liaw CC, Huang HC, Hsiao PC, Zhang LJ, Lin ZH, Hwang SY, Hsu FL, Kuo YH. 5β, 19-epoxycucurbitane triterpenoids from Momordica charantia and their anti-inflammatory and cytotoxic activity. *Planta Medica.* 2014 Dec 3:62-70. [Crossref], [Google Scholar], [Publisher]
7. Egberg MD, Kappelman MD, Gulati AS. Improving care in pediatric inflammatory bowel disease. *Gastroenterology Clinics*. 2018 Dec 1;47(4):909-19. [Crossref], [Google Scholar], [Publisher]
8. Crowley E, Muise A. Inflammatory bowel disease: what very early onset disease teaches us. *Gastroenterology Clinics.* 2018 Dec 1;47(4):755-72. [Crossref], [Google Scholar], [Publisher]
9. Coward S, Kaplan GG, IBD in the new world, old world, and your world. *Inflammatory Bowel Disease: Diagnosis and Therapeutics*, 2017;13-27. [Crossref], [Google Scholar], [Publisher]
10. Chang M, Chang L, Chang HM, Chang F. Intestinal and extraintestinal cancers associated with inflammatory bowel disease. *Clinical Colorectal Cancer*. 2018 Mar 1;17(1): e29-37. [Crossref], [Google Scholar], [Publisher]
11. De Souza HS, Fiocchi C, Iliopoulos D, The IBD interactome: an integrated view of aetiology, pathogenesis and therapy. *Nature Reviews Gastroenterology & Hepatology*, 2017;14:739-749. [Crossref], [Google Scholar], [Publisher]
12. Wirtz S, Popp V, Kindermann M, Gerlach K, Weigmann B, Fichtner-Feigl S, Neurath MF, Chemically induced mouse models of acute and chronic intestinal inflammation. *Nature Protocols*, 2017; 12:1295-1309. [Crossref], [Google Scholar], [Publisher]
13. Al-Laith AA, Alkhuzai J, Freije A. Assessment of antioxidant activities of three wild medicinal plants from Bahrain. *Arabian Journal of Chemistry.* 2019 Dec 1;12(8):2365-71. [Crossref], [Google Scholar], [Publisher]
14. Tian T, Wang Z, Zhang J, Pathomechanisms of oxidative stress in inflammatory bowel disease and potential antioxidant therapies. *Oxidative Medicine and Cellular Longevity*, 2017;2017. [Crossref], [Google Scholar], [Publisher]
15. Mohammed A, Tajuddeen N. Antidiabetic compounds from medicinal plants traditionally used for the treatment of diabetes in Africa: A review update (2015–2020). *South African Journal of Botany.* 2022 May 1; 146:585-

602. [Crossref], [Google Scholar], [Publisher]
16. Zhu QL, Guo SN, Wen F, Zhang XL, Wang CC, Si LF, Zheng JL, Liu J. Transcriptional and physiological responses of Dunaliella salina to cadmium reveals time-dependent turnover of the ribosome, photosystem, and ROS-scavenging pathways. *Aquatic Toxicology*. 2019 Feb 1; 207:153-62. [Crossref], [Google Scholar], [Publisher]
17. Kim SK, Jung J, Jung JH, Yoon N, Kang SS, Roh GS, Hahm JR. Hypoglycemic efficacy and safety of Momordica charantia (bitter melon) in patients with type 2 diabetes mellitus. *Complementary Therapies in Medicine*. 2020 Aug 1; 52:102524. [Crossref], [Google Scholar], [Publisher]
18. Wang S, Li Z, Yang G, Ho C.T, Li S, Momordica charantia: a popular health-promoting vegetable with multifunctionality. *Food & Function*, 2017;8:1749-1762. [Crossref], [Google Scholar], [Publisher]
19. Opstelten JL, de Vries JH, Wools A, Siersema PD, Oldenburg B, Witteman BJ. Dietary intake of patients with inflammatory bowel disease: A comparison with individuals from a general population and associations with relapse. *Clinical Nutrition*. 2019 Aug 1;38(4):1892-8. [Crossref], [Google Scholar], [Publisher]
20. Håkansson, Å., Tormo-Badia, N., Baridi, A., Xu, J., Molin, G., Hagslätt, M. L., ... & Ahrné, S. (2015). Immunological alteration and changes of gut microbiota after dextran sulfate sodium (DSS) administration in mice. *Clinical and Experimental Medicine*, *15*, 107-120. [Crossref], [Google Scholar], [Publisher]

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