

Safety Assessment and Prophylactic Efficacy of *Moringa stenopetala* Leaf Extract through Mitigation of Oxidative Stress in BV-2 Microglial Cell

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Abstract

Moringa stenopetala is often consumed as food and used in folkloric medicine for the management of several diseases.

Purpose: This study was set up in order to assess the effect of aqueous extract of *Moringa stenopetala* on cell viability and oxidative stress biomarkers in BV-2 microglial cells. Aqueous extracts of *M. stenopetala* were prepared, lyophilized and reconstituted in 0.5% dimethylsulphoxide (DMSO). Cells were treated with *M. stenopetala* extracts (0.1 - 100 µg/ml) for cell viability and nitric oxide (NO) production tests. However, *M. stenopetala* extract (50 µg/ml) was used in the treatment of cells for the determination of protein carbonyl content and reactive oxygen species (ROS) level. Incubation of BV-2 microglia cell with *M. stenopetala* extract maintained cell viability, diminished NO and ROS levels, and reduced protein carbonyl contents. Chlorogenic acid, rutin, kaempferol and quercetin derivatives were the main phenolic compounds identified in *M. stenopetala* leaf extract. These phenolic compounds present in *M. stenopetala* may be responsible for the mitigation of oxidative stress in BV-2 microglial cells.

Introduction

Microglial cells are immune cells found in the CNS and key mediators of neuroinflammation (Bernhardi et al. 2015). Microglial cells play crucial significance in the balance between brain injury and diseases. They are the major phagocytic cell of the brain and their main role is to eradicate cells targeted for apoptosis thus contributing immensely to oxidative processes in the central nervous system (CNS) (Loane and Kumar 2016). The brain contains low level of antioxidants, making it vulnerable to pro-oxidants. Thus, activation of microglial cells can occur as a result of any type of pathological insult to the brain. This can lead to the release of pro-inflammatory substances such as nitric oxide (NO), tumor necrosis factor-alpha (TNF-α), and interleukin 6 (IL-6) (Hanisch and Kettenmann 2007).

In physiological conditions, the microglia exhibit low levels of reactive oxygen species (ROS) in the system. However, an assault can lead to a rapid rise in ROS levels (Simpson and Oliver 2020). These ROS can then proceed as second messengers triggering signaling pathways which could lead to the activation of pro-inflammatory transcription factors such as the nuclear factor kappa B (NF-κB), as well as pro-inflammatory mediators such as inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), and IL-6 (Morgan and Liu 2011). The main production of ROS in the microglia occurs via the NADPH oxidoreductase (NOX) system (Block and Hong, 2005). NOX is a multi-subunit enzyme complex which transfers oxygen from NADPH to molecular oxygen resulting in the formation of the superoxide anion (Fischer et al. 2012). Activation of microglia also leads to a rise in the generation of nitric oxide (NO). NO is produced from L- Arginine by the action of iNOS, which produces high levels of NO which may cause neuronal death via inhibition of mitochondrial cytochrome oxidase in neurons as well as mitochondrial respiration (Cunningham et al. 2005).

Moringa species are highly nutritious which benefits people in terms of providing daily nutritional supplements and boosting their immune systems (Popoola and Obembe 2013). It is a perennial angiosperm and edible plant, and is described often as the miracle tree, tree of life, and God's Gift to man coloration (Mekonnen 2002). The traditional uses of genus Moringa include healing fever, asthma, diarrhea, skin infections, sore throats, wounds, and anxiety. The seeds of Moringa are used for purifying water, the leaves as nutrition supplements, the oil as a biofuel, the trunks as gum, the flowers as honey, and all of the plant parts can also be used for medicinal purposes (Fahey 2005). *Moringa stenopetala* (Baker f.) Cufod. belongs to the family Moringaceae and order Brassicales (Mekonnen 2002). It is grown in various regions of the world, especially in Africa, Asia and South America (Rani et al. 2018). The leaves and seeds of *M. stenopetala* are commonly consumed as spices, vegetables, salad and used as a nutritional supplement worldwide, as it encapsulates significant amounts of vitamins, minerals, amino acids, lipids and various phytochemicals (Rani et al. 2018). A wide variety of nutritional and medicinal virtues have been attributed to its roots, bark, and leaves, flowers, fruits, and seeds (Mekonnen and Drager, 2003; Seid, 2013; Rani et al. 2018). Phytochemicals such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids, glucosinolates, isothiocyanates, and glycerol-1-9-octadecanoate, have been reportedly found in the plant (Mekonnen and Drager 2003). Most of its biological activity is attributed to its high level of flavonoids, glycosides, and glucosinolates (Mekonnen and Drager 2003). The high oleic acid content and high stability of *M. stenopetala* and *M. oleifera* seed oil makes it suitable to use as edible oil, cosmetic oil, biodiesel, and lubrication oil for machinery and watches (Rashid et al. 2008). *M. stenopetala* has bigger and wider leaf than *M. oleifera*. *M. stenopetala* and other *Moringa spp.* have been reported to exhibit antimicrobial, antioxidant, antihyperglycemic, antihypertensive, erectogenic, cytotoxic and anticancer properties (Seid 2013; Habtemariam 2015; Adefegha et al. 2017; 2019; 2021). The effect of *M. stenopetala* extract on crucial biochemical parameter in BV-2 microglia cells has not been reported in literature, considering the oxidative damage and inflammatory processes associated with neuronal cells especially the microglia cells. Therefore, this study seeks to investigate the effect of *M. stenopetala* extract on the viability of BV-2 microglial cells, the prophylactic efficacy of the extract on BV-2 microglial cells as well as identify compounds with neuroprotective potentials from the plant.

Materials And Methods

Chemicals and Reagents

All chemicals, reagents and media used in the cell culture experiments were of analytical grade. They were bought from Kasvi (São José dos Pinhais, PR, Brazil), Corning Inc. (Corning, NY, USA), and Vitrocell Embriolife (Campinas, SP, Brazil).

Statement on Plant Collection, Identification and Authentication

All relevant institutional, national, and international guidelines and legislation were strictly adhered to for experimental research and field studies on plants. *Moringa stenopetala* leaves were gotten from the

Medicinal garden of the Federal University of Technology, Akure, Nigeria. Identification of the plant was done by Mr. B. E. Omomoh of the Department of Forestry and Wood Technology, Federal University of Technology, Akure, Nigeria with further authentication achieved by a taxonomist, Dr. S. L. Fayeun of the Department of Crop, Soil and Pest management, Federal University of Technology, Akure, Nigeria. A voucher sample of *Moringa stenopetala* leaves (MS081) was deposited in the University herbarium.

Extraction and Preparation of *Moringa stenopetala*

They were cleaned, rinsed under running water, air dried and pulverized. Aqueous extraction was done according to the method previously described by Adefegha et al. (2021). One hundred gram of *Moringa stenopetala* leaves was soaked into boiled water (100°C) for hot water extraction. It was allowed to cool for 15 mins while the extraction was aided by continuous stirring using a magnetic stirrer. It was subsequently filtered and the filtrate was refrigerated for 48 h. Lyophilization was done for 72 h at -43 °C for 150 mmHg to obtain the dried extract used for subsequent analysis. *Moringa stenopetala* leaf extract doses ranging from 0.1 µg/mL to 100 µg/mL) were calculated and prepared by dissolution in 0.5% dimethylsulphoxide (DMSO) and used for this *in vitro* study.

Incubation of BV-2 microglial cell culture

The mouse brain BV-2 microglial cell line used for the experiment was purchased from the Rio de Janeiro Cell Bank (BCRJ, Rio de Janeiro, RJ, Brazil). The mouse brain BV-2 microglial cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing 4500 mg/L glucose, 1500 mg/L sodium bicarbonate, 2 mM L-glutamine, and 1 mM sodium pyruvate. The medium was supplemented with 10% fetal bovine serum (FBS) to a final concentration and supplemented with 1% penicillin/streptomycin (10.000 U/ml; 10 mg/ml). . All cell incubations were performed at a temperature of 37°C, kept in 5% CO₂ and maintained at 95% relative humidity. Suspended BV-2 microglia cultured cells were seeded at 3 million cells/Petri dish (surface area 20 cm³) in 5 mL medium. After 25 min of incubation, loosely adherent cells were removed by tapping the sides of the dish, followed by two washes in PBS. After overnight incubation, cells were washed once in PBS, followed by addition of 5 ml medium containing 10% FBS.

Preparation of *Moringa stenopetala*

The leaves of *M. stenopetala* were obtained from the Medicinal garden of the Federal University of Technology, Akure, Nigeria. They were handpicked to remove dirt, washed with running water, air dried and ground into powder. Aqueous extraction was done by adding 2l of boiling water to 100 g of each of these leaves, and each mixture was placed on a magnetic stirrer, rotated for 15 min. and subsequently filtered. The filtrate was refrigerated for 48 hours and lyophilized within 72 hours at -43 °C for 150 mmHg to obtain the dried extract used for subsequent analysis. Various doses of *M. stenopetala* extracts ranging from 0.1 µg/ml to 100 µg/ml) were prepared by dissolution in 0.5% dimethylsulphoxide (DMSO) and used in this study.

BV-2 microglial cell culture and treatment

Cells were subsequently treated with *M. stenopetala* extracts (0.1, 0.5, 1, 2.5, 5, 7.5, 10, 20, 50 and 100 µg/ml) for cell viability and nitric oxide production assays. However, in other experiments, 50 µg/ml of *M. stenopetala* extracts was used for the treatment of cells.

Cell viability assay

The viability of BV-2 microglial cells was assessed in accordance with previous method described by Sylvester (Sylvester, 2011) with slight modification. BV-2 microglial cells (2×10^5 cells/ml, 96-well plate) were allowed to adhere and grow overnight. Cells were then incubated in 2% FBS-containing medium, or treated with dimethyl sulfoxide (DMSO) or different concentrations of *M. stenopetala* extract (0.1 - 100 µg/mL) for 6 hours and 24 hours. After incubation, 2 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent replaced the medium and was incubated in a 5% carbon dioxide (CO₂) incubator at 37°C for an additional 4 hours. The BV-2 cells were then harvested with 50 µl of dimethyl sulfoxide (DMSO) and transferred to 96-well plates. The absorbance was measured at 540 nm using a microplate reader.

NO analysis

NO was evaluated by measuring the amount of nitrite in BV-2 microglial cells (2×10^5 cells/ml, 96-well plate), using Griess reagent (0.1% naphthylethylenediamine, 1% sulfanilamide in 2.5% H₃PO₄) according to the modified method of Yoo et al. (2012). BV-2 microglial cells were grown on 96-well plates and treated with medium or DMSO or different concentrations of *Moringa stenopetala* extract (0.1 - 100 µg/ml) for 6 hours and 24 hours. The production of NO was determined basing on the Griess reaction. In short, 50 µl of culture supernatant was allowed to react with an equal volume of Griess reagent in 96-well plates for 10 min. at room temperature in the dark. The absorbance at 550 nm was determined using a microplate reader. NO was subsequently calculated as % Control.

Cell Morphology

The cell morphology was assessed according to the method described by Wang et al. (2014). BV-2 microglial cells were placed on 96 well plates and medium or *Moringa stenopetala* leaf extract (50 µg /ml) was subsequently added. The mixture was incubated for 24 hours and the photomicrographs of the culture plates were captured using converted light microscopy.

Determination of Protein Carbonyl

Oxidative damage to proteins was determined in BV-2 microglial cells (2×10^5 cells/ml, 96-well plate) treated with medium (Control) or 50 µg/mL of *Moringa stenopetala* extract for 24 hours based on the formation of protein-hydrazone as a result of the reaction between 2,4-dinitrophenylhydrazine (DNPH) and protein carbonyls using the Cayman's Protein Carbonyl Assay kit (Weber et al. 2015). Absorbance of

the samples was measured at 370 nm using the microplate reader. Carbonyl content was determined using the extinction coefficient of DNPH ($0.022 \mu\text{M}^{-1}\text{cm}^{-1}$). The total protein was then measured at 540 nm using Bradford method (Bradford, 1976).

Reactive oxygen species measurement by 2',7'- dichlorofluorescein diacetate dye

Reactive oxygen species (ROS) level was determined in BV-2 microglial cells (2×10^5 cells/ml, 96-well plate) treated with medium (Control) or 50 $\mu\text{g}/\text{ml}$ of *Moringa stenopetala* extract (50 $\mu\text{g}/\text{mL}$) according to the modified method described by Ali et al. (1992). Briefly, the seeded cell mixture was diluted (1:10) with 10 mM Tris buffer (pH 7.4) and loaded with 5 μl of 2',7'-dichlorofluorescein diacetate (DCFH-DA) in methanol at 37 °C in 5 % CO_2 for 15 min, to form a non-fluorescent compound, 2',7'-dichlorofluorescein (DCFH). Fluorescence was recorded prior and after an additional 60-min period of incubation. The formation of a fluorescent oxidized derivative of DCFH named 2',7'-dichlorofluorescein (DCF) was measured at an excitation wavelength of 488 nm and an emission wavelength of 525 nm on a LS-50 spectrofluorimeter. The cuvette holder was thermostatically maintained at 37 °C. Auto fluorescence was checked by the inclusion of blank samples (unloaded tissue fraction), and it was always less than 10 % of the total fluorescence. ROS formation was quantified from a DCF standard curve in methanol (0.05 – 1.0 μM).

Quantification of phenolic compounds by HPLC-PDA

Phenolic compounds from *Moringa stenopetala* were analyzed by HPLC coupled with photodiode array (PDA) detector using a reverse-phase C-18 Hypersil Gold column (5 μm particle size, 150 mm, 4.6 mm) following the method described by Quatrin et al. (2019). Injection volume was 20 μL and the mobile phases were composed of 5% (v/v) methanol in acidified water (0.1% (v/v) of formic acid) and 0.1% (v/v) of formic acid in acetonitrile. The chromatograms for quantification purposes were obtained at 280 nm, for hydroxybenzoates, at 320 nm for hydroxycinnamates, and at 360 nm for flavonols. The phenolic compounds from samples were identified by comparison with the retention time of authentic standards and the spectral data obtained from UV–visible absorption spectra. Stock solution of standard references was prepared in the initial mobile phase and was diluted at equidistant points within the concentration range of LOQ–60 mg L^{-1} . Calibration curve for catechin: $y = 19861x + 21544$ ($r=0.997$); chlorogenic acid: $y = 90494x + 49749$ ($r=0.997$) and rutin: $y = 53524x + 41979$ ($r=0.978$). The limit of detection (LoD) and limited of quantification (LoQ) for catechin, chlorogenic acid and rutin were, respectively, 0.040 and 0.122 ppm; 0.015 and 0.047 ppm; and 0.099. Compounds that are derivatives of one of the standard monomers were quantified by equivalence and results were expressed as mg per 100 g of dry sample weight.

Statistical Analysis

Each result is an averaged value obtained from at least six independent experiments, and is expressed as mean $\pm$ standard error of the mean (SEM). Statistical significance was determined using one-way analysis of variance followed by Dunnett's test, after comparing each treated group and the Control group. Statistical significance was defined as * $p < 0.05$.

Results And Discussion

Microglial cells play major roles in response to immunological stimuli and brain injury, which also contribute to inflammation-mediated neurodegeneration (Lull and Block, 2010). In this study, the dye 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) was used to quantify the viability in BV-2 microglial cell (Henn et al. 2009; van Tonder et al. 2015). The non-significant ($p > 0.05$) difference in cell viability as indicated by the MTT assay between the cells treated with medium, DMSO, various concentration of *M. stenopetala* for 6 hours (Fig. 1b), suggest that the *M. stenopetala* extract at the various doses tested (0–100 $\mu\text{g/mL}$) were not cytotoxic to the BV-2 microglia cells. In a similar manner, after 24 hours, the extracts of *M. stenopetala* (0–50 $\mu\text{g/ml}$) was not cytotoxic to the BV-2 microglia cells (Fig. 1a & 1b). However, at the end of 24 hours, treatment of *M. stenopetala* (100 $\mu\text{g/ml}$) with BV-2 microglia cells was observed to be cytotoxic to the cell. This is suggestive that *M. stenopetala* extract doses may toxic to the cell at the concentration of 100 $\mu\text{g/ml}$ and above (Fig. 1b). This agrees with previous study where extracts from *Moringa stenopetala* were reported to be cytotoxic in HEPG2 cells (Mekonnen et al. 2005).

Normal levels of nitric oxide (NO) act as a neuromodulator at synaptic junctions (Habib and Ali 2011). Increasing NO levels may lead to neuronal respiration inhibition and glutamate release in microglia cells, which might lead to the excitotoxic death of neurons and tissue damage (Cho et al. 2016). Thus, high levels of NO secreted by activated microglia induce oxidative stress and inflammatory processes in the neuron, thus contributing to neurodegenerative disorders (Cho et al. 2016; Oh et al. 2019). The inhibitors of NO production have been considered as potential anti-inflammatory agents (Oh et al. 2019). As observed in Fig. 2, no significant ($p > 0.05$) difference in NO production of the cells incubated with the medium (Control), DMSO (DMSO Control) and varying concentrations of *M. stenopetala* extracts (0.1–100 $\mu\text{g/mL}$) for 6 hours (Fig. 2a). Furthermore, at 24 hours of incubation of BV-2 microglial cells with *M. stenopetala* extracts (0.1–100 $\mu\text{g/ml}$), the NO production was decreased significantly in the different BV-2 microglial cells treatment (medium or DMSO or 0.1–50 $\mu\text{g/ml}$ extract of *M. stenopetala*) (Fig. 2b). Furthermore, cells treated with 100 $\mu\text{g/ml}$ of *M. stenopetala* extracts were observed to exhibit significantly ($p < 0.05$) elevation in NO production when compared to the Control or DMSO control. In this study, we observed decrease in NO production after 24-hour treatment of BV-2 microglia cell with *M. stenopetala* extract (0–50 $\mu\text{g/ml}$). Our report supports the assertion that the inhibition of NO production in BV-2 cells is an effective treatment for inflammation in the CNS (Cho et al. 2016; Oh et al. 2019). Hence, *M. stenopetala* extract may represent interesting sources of anti-inflammatory agents. Although, the mechanism(s) by which *M. stenopetala* extract extract decreases NO in 24 h but not in 6 h are unclear (Fig. 2a and Fig. 2b), the observed results may be attributed to the antioxidative effects of *M. stenopetala* extract as typified by its radical scavenging, metal chelating and reducing abilities (Habtemariam 2015; Tesfaye and Libsu 2015).

Based on the results obtained from the cell viability and NO production assays, the photomicrographs of the culture plates treated with the medium or *M. stenopetala* leaf extract (50 $\mu\text{g/ml}$) for 24 hours, were taken using converted light microscopy cells. Similarities in the cell fragments of BV-2 microglial treated

with *M. stenopetala* extract (Fig. 3). No morphological change was observed and this suggest that the membrane integrity was maintained upon the administration of 50 µg/ml of MS extract on BV-2 microglial cell. This could be an indication that the microglial cells retain its phenotype after incubation with 50 µg/mL of *M. stenopetala* extract (Wang et al. 2014).

Among glial cells, microglia are the primary immune cell of the brain and function to protect the central nervous system (CNS) from injury and invading pathogens (Jeong et al. 2019). Oxidative stress-induced by neuronal death results in the production of reactive oxygen species (ROS) leading to an imbalance of oxidant production and antioxidant defense (Di Meo et al., 2016). Therefore, ROS is well known as key pathophysiological and physiological mechanisms in activated microglial cell and brain injury (Jeong et al. 2019; Di Meo et al. 2016). As shown in Fig. 4, there was a significant ($p < 0.05$) reduction in reactive oxygen species (ROS) production of cells treated with *M. stenopetala* for 24 hours when compared to that of the Control. The observed decrease in ROS production in BV-2 microglia cells treated with *M. stenopetala* may be attributed to the antioxidant properties of the *M. stenopetala* *in vivo* and *in vitro* (Yang et al. 2006).

The protein oxidative changes involving amino acid side chain give rise to a carbonyl group. Such modification could be detected in cells. The protein carbonyl content may therefore be used as a biological marker of oxidative stress (Davies 2016). Significant ($p < 0.05$) decrease in protein carbonyl of cells incubated for 24 hours with *M. stenopetala* as compared to that of the control was observed (Fig. 5). The reduced protein carbonyl level of BV-2 microglia cells incubated with *M. stenopetala* extract could indicate decreased oxidation of protein that may have been facilitated by the chemical constituents of the plant.

The HPLC-PDA characterization of phenolic compounds in *M. stenopetala* extracts indicates that hydroxybenzoic acid derivatives at 280 nm had phenolic contents of 616.5 ± 18.6 mg/100 g of dry weight) and accounts for about 12.9% of the total phenolic compound. The total sum of the hydroxycinnamic acid derivatives at 320nm was about (822.0 ± 20.4 mg/100 g of dry weight, accounting for 17.2%. In addition, flavonol derivatives were detected at 360 nm and rutin was observed to be present in highest quantity as it accounts for about 67.4% of the total phenolic compounds found in *M. stenopetala*. Other phenolic compounds identified and quantified include chlorogenic acid, quercetin derivatives and kaempferol (Fig. 6 and Table 1). These phenolic compounds and their derivatives may have contributed towards the prophylactic efficacy and antioxidative effect of *M. stenopetala* in BV-2 microglial cell.

Conclusion

The prophylactic efficacy through the antioxidative effects of *M. stenopetala* extract could be attributed to the presence of the phenolic compounds as well as its ability to maintain cell viability, inhibit microglia-mediated NO production, reduce ROS level and diminish protein carbonyl content. Thus, we can infer that the potential therapeutic effect of the phenolic compounds found in *M. stenopetala* may be responsible

for the maintenance of cell viability in BV-2 microglia cells, and prevention of oxidative stress in normal cells. This study further proves the prophylactic efficacy and antioxidative properties of *M. stenopetala* in normal cells.

Abbreviations

central nervous system (CNS), 2',7'-dichlorofluorescein diacetate (DCFH-DA), 2',7'-dichlorofluorescein (DCFH), dimethylsulphoxide (DMSO), 2,4-dinitrophenylhydrazine (DNPH), inducible nitric oxide synthase (iNOS), interleukin-6 (IL-6), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), NADPH Oxidoreductase (NOX), nitric oxide (NO), nuclear factor kappa B (NF-KB), reactive oxygen species (ROS); Roswell Park Memorial Institute (RPMI), tumor necrotic factor -alpha (TNF-α)

Declarations

Conflict of interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Data Availability

The data of this study was generated in the laboratory after a cautious and diligent experimental work. The data that support the findings of this study are available from the corresponding author, (Dr. S. A. Adefegha), upon reasonable request.

Authors' Contribution

Stephen Adeniyi Adefegha carried out cell culturing, performed the experiments, analyzed data and drafted the manuscript; Ademir F. Morel was involved in the preparation of extracts and participated in the cell based experiments; Ivana Beatrice Manica Da Cruz provided the BV-2 microglial cells and analyzed cell morphology; Vitor Mostardeiro conducted the culturing of cells, performed experiments and analyzed data; Vera Maria Morsch provided resources, designed and supervised the experiments. Sabrina Somacal carried out the phenolic characterization of *Moringa stenopetala* leaves, Maria Rosa Chitolina Schetinger was involved in the conceptualization and proof reading of the manuscript. All the listed authors have contributed to the writing and editing of the manuscript.

Instruments

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Tables

Table 1
Quantification of phenolic compounds of *Moringa stenopetala* extract.

	λ (nm)	Quantification (mg/100 g of dry weight)*	%
<i>Hydroxybenzoate derivatives (HBD)</i> 280 nm			
Sum of non-identified HBD		616.5 $\pm$ 18.6	12.9
Total HBD		616.5 $\pm$ 18.6	12.9
<i>Hydroxycinnamate derivatives (HCD)</i> 320 nm			
Chlorogenic acid		208.2 $\pm$ 22.0	4.4
Sum of non-identified HCD		613.8 $\pm$ 1.5	13.4
Total HCD		822.0 $\pm$ 20.4	17.2
<i>Flavonol derivatives</i> 360 nm			
Rutin		3215.4 $\pm$ 24.9	67.4
Kaempferol		64.4 $\pm$ 2.1	1.4
Quercetin derivative		35.8 $\pm$ 2.1	0.8
Sum of non-identified flavonols derivatives		16.8 $\pm$ 1.7	0.4
Total flavonol derivatives		3332.3 $\pm$ 27.4	69.8
Total phenolic compounds		4770.9 $\pm$ 25.6	100.0
Results were expressed as mean $\pm$ standard deviation. *Hydroxybenzoate derivatives were quantified as equivalent to catechin; hydroxycinnamate derivatives were quantified as equivalent to chlorogenic acid; flavonols were quantified as equivalent to rutin.			

Figures

Figure 1a

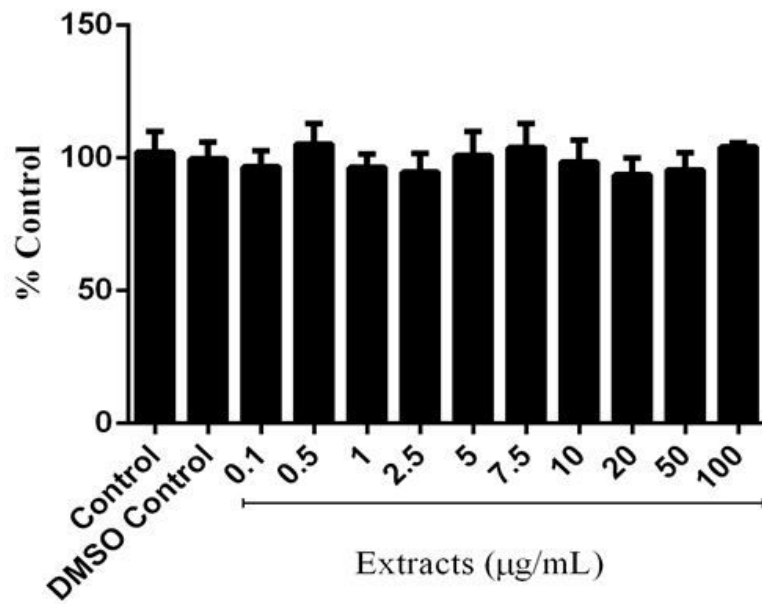


Figure 1b

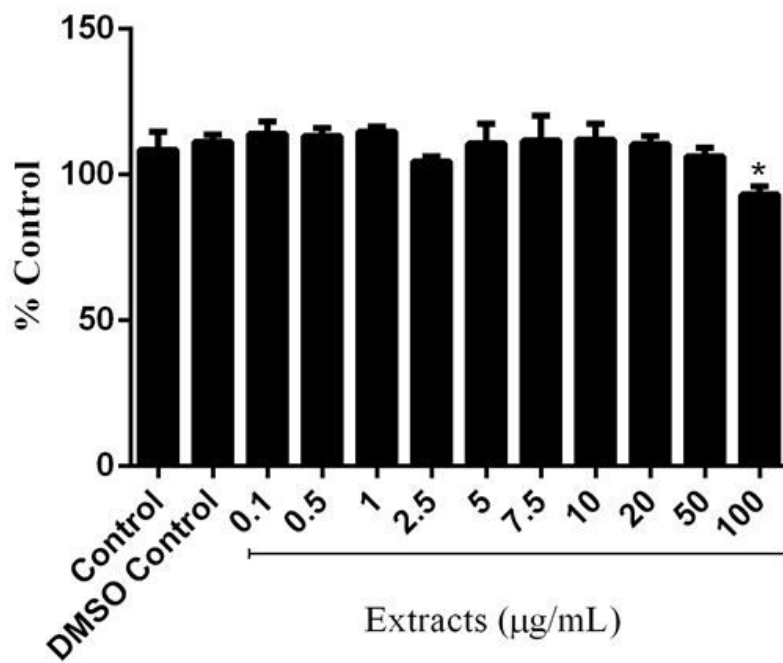


Figure 1

1a. Effect of *Moringa stenopetala* leaf extract on the cell viability of BV-2 microglial cells for 6 h. No significant difference ($p > 0.05$) between the cells treated *M. stenopetala* extract doses and the Control cells.

1b. Effect of *Moringa stenopetala* leaf extract on the cell viability of BV-2 microglial cells for 24 h. No significant difference ($p > 0.05$) between the cells treated *M. stenopetala* extract doses and the Control cells. Statistical significance was defined as $* p < 0.05$ for cell treated *M. stenopetala* extract dose and the Control cell.

Figure 2a

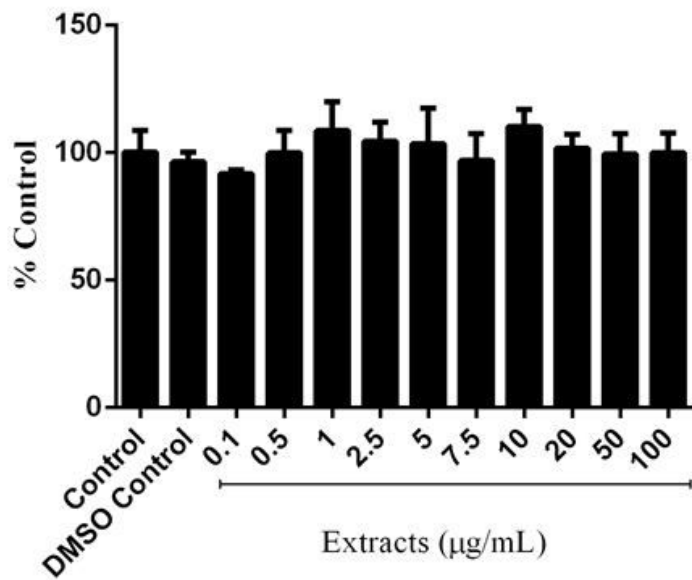


Figure 2b

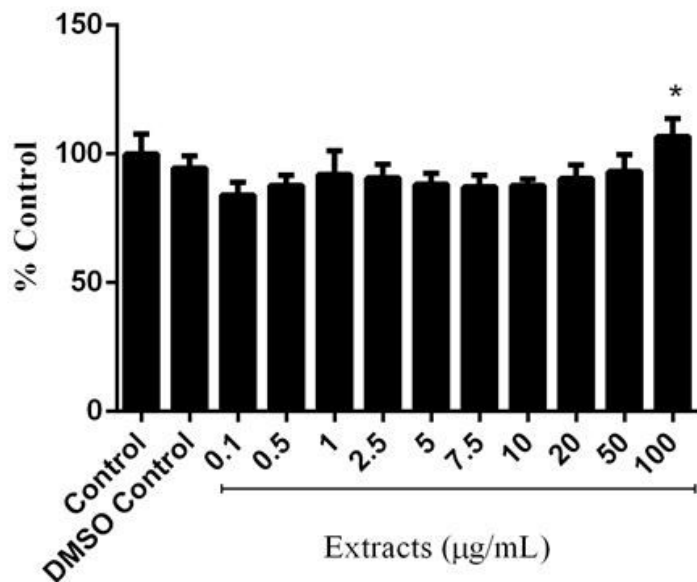


Figure 2

2a. Effect of *Moringa stenopetala* leaf extract on Nitric oxide production of BV-2 microglial cells for 6 h. No significant difference ($p > 0.05$) between the cells treated *M. stenopetala* extract doses and the Control cells.

2b. Effect of *Moringa stenopetala* leaf extract on Nitric oxide production of BV-2 microglial cells for 24 h. No significant difference ($p > 0.05$) between the cells treated *M. stenopetala* extract doses and the Control cells. Statistical significance was defined as * $p < 0.05$ for cell treated *M. stenopetala* extract dose and the Control cell.

Control



M. stenopetala



Figure 3

Morphology of BV-2 Microglial Cells Incubated with *Moringa stenopetala* leaf extract for 24 hours.

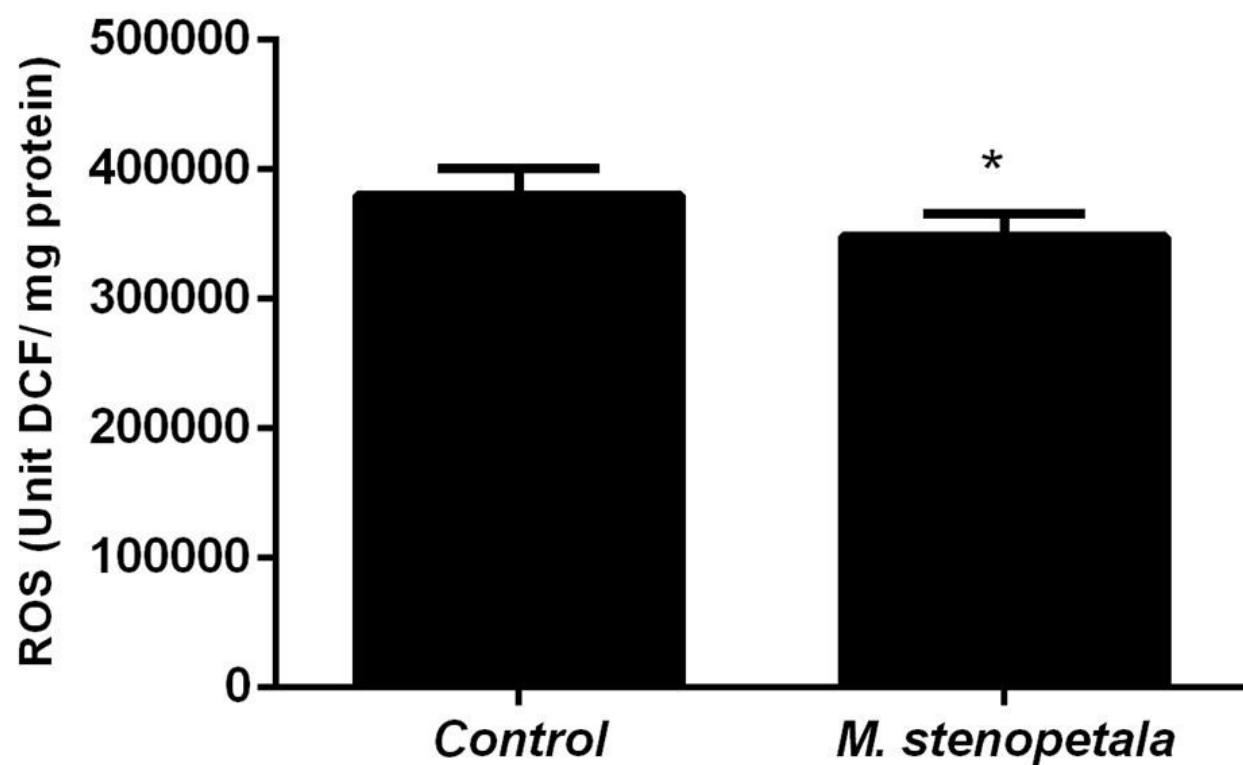


Figure 4

Effect of *Moringa stenopetala* leaf extract on reactive oxygen species of BV-2 microglial cells after 24 h incubation. Statistical significance was defined as * $p < 0.05$ for cells treated *M. stenopetala* extract dose and the Control cell.

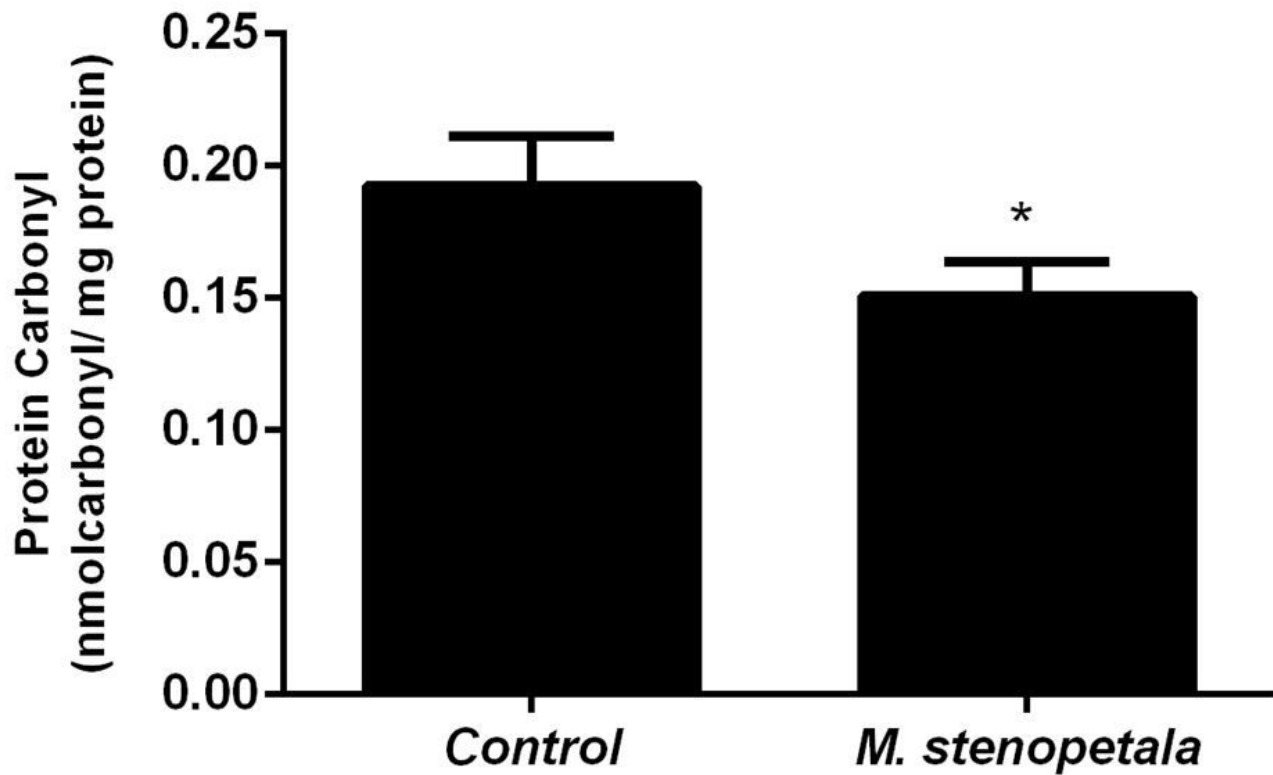


Figure 5

Effect of *Moringa stenopetala* leaf extract on protein carbonyl levels of BV-2 microglial cells after 24 h incubation. Statistical significance was defined as * $p < 0.05$ for cell treated *M. stenopetala* extract dose and the Control cell.

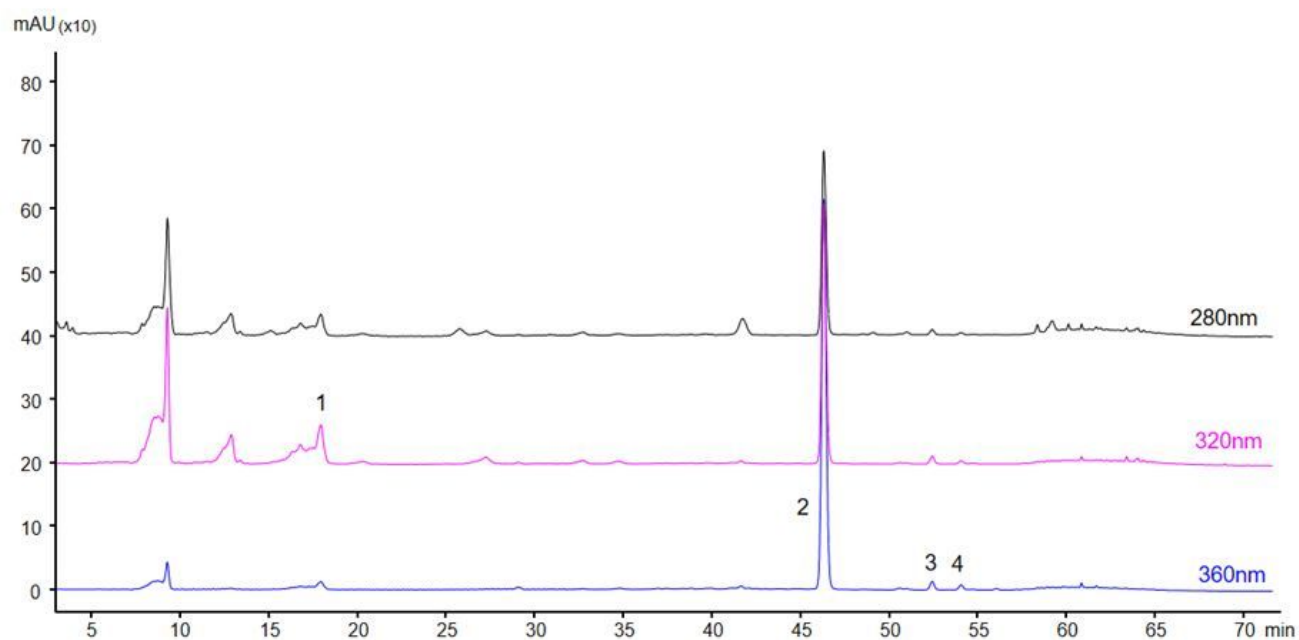


Figure 6

Representative chromatogram of phenolic compounds from *Moringa stenopetala* extract. Chromatograms were acquired at 280 nm, 320 nm, and 360 nm. Peak 1: Chlorogenic acid; Peak 2: Rutin; Peak 3: Quercetin pentoside; Peak 4: Kaempferol derivative