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An Ethyl Acetate Fraction of *Moringa oleifera* Lam. Inhibits Human Macrophage Cytokine Production Induced by Cigarette Smoke

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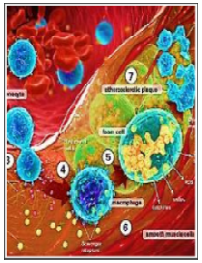
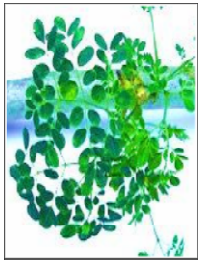
Background and Problems

Cigarette smoke generates highly reactive oxygen and nitrogen species as well as free radicals, leading to oxidative stress and damage of the lung and even the whole body.



Monocytes and tissue macrophages response to stress, resulting in the activation of NF-κB-dependent pro-inflammatory genes and cytokines expression.



Polyphenolic compound found in *Moringa oleifera* Lam. proposed to have an antioxidant activities as well as anti-inflammatory activity.

The useful medicinal plant *Moringa oleifera* Lam. may provide an essential health benefit for cigarette smoker or people who exposed to oxidative stress.

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Moringa oleifera Lam.

Small – medium sized tree, 10-12 m. in height

Found in tropical and sub-tropical climates

Drought tolerance, can grow in various physical conditions

Widespread cultivation over 70 countries

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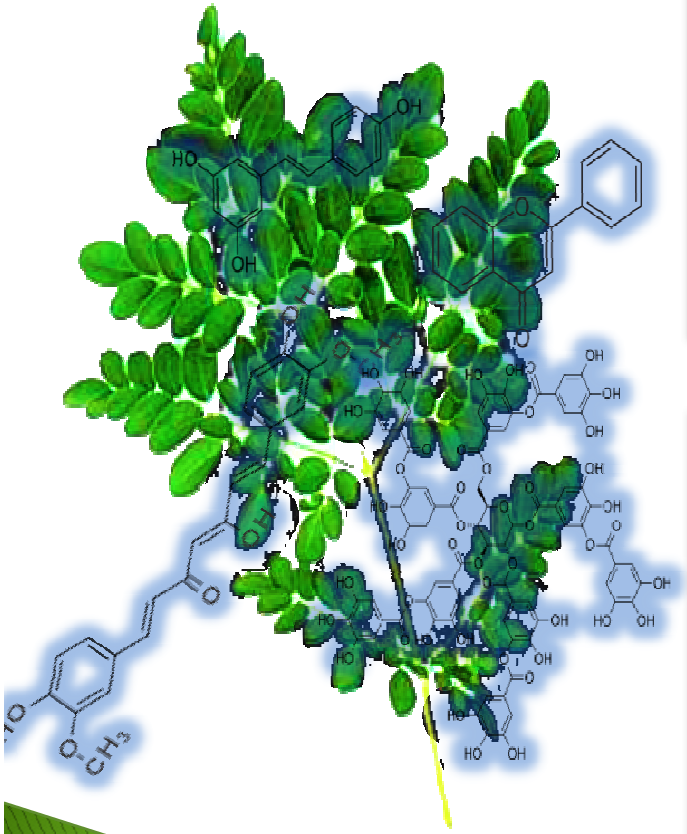
Insight into the Activities of *Moringa*



Moringa oleifera leaves contains a wide variety of phenolic compounds

- Monophenol, Polyphenol, Phenolic acid, etc.

The compounds are responsible for the biological activities of *Moringa*

- Antioxidant, Antimicrobial, Anti-inflammation, etc.

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Aim

- To study the effect of *Moringa oleifera* Lam. leaves extract on cigarette smoke-induced cytokines expression in human monocyte-derived macrophages.

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Experimental Design

Extraction and Fractionation of Fresh *Moringa oleifera* Lam. Leaves

↓

Determination of Phenolic Content and Antioxidant Activities

↓

Cytotoxicity Testing on human Monocyte-derived Macrophages (MOEF, ASA, NIC, CSE)

Treatment of *Moringa oleifera Lam.* Extract on human Monocyte-derived Macrophages

↓

LPS or CSE Stimulation on Treated Cells

↓

Pro-Inflammatory Genes and Transcription Factor.

↓

Cytokines Associated in Pro-Inflammatory Response

Methods and Results

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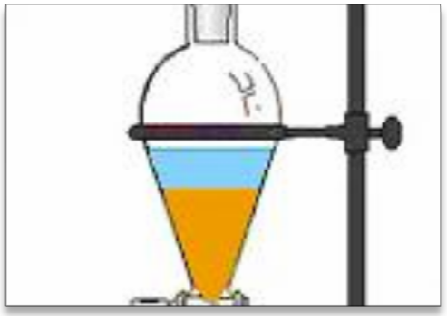
Preparation of *Moringa* Extract and Fractions

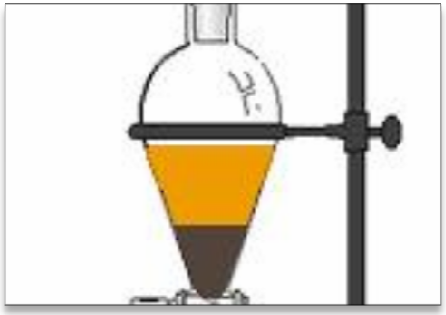
Extract fresh *Moringa* leaves with 50% methanol + 1% acetic acid

Filter and concentrate by evaporating at 40 °C

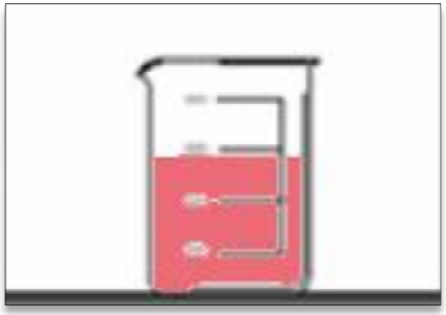


Partition with distilled water and diethyl ether

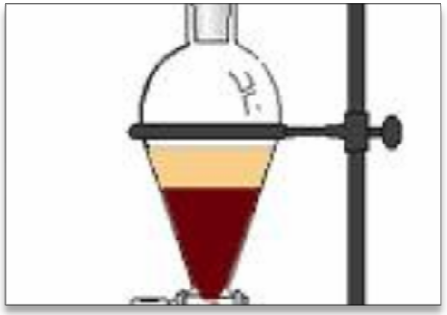
Adjust pH of aqueous part to 8.5 to protein denature and convert of phenolic acid



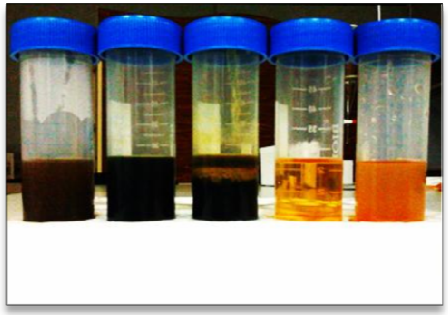
Partition with chloroform to separate non-phenolic



Adjust pH to 3.5 to change phenolic sodium salt to phenolic acid



Extract with ethyl acetate to fully fractionate polyphenol form



Obtain the extract and fractions

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Determination of Total Phenolics

Folin-Ciocalteu Method

- Determination of total phenolic compounds in sample
- Based on the ability of phenolic-OH group to convert a yellow phosphomolibdic-phosphotunstic complex to blue colour
- Express as milligram Pyrogallol equivalent per gram of dry extract (mg PyE/g)

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Determination of Free Radical Scavenging Activity

ABTS Radical Cation Decolorization Assay

- Determination of total antioxidant of the sample
- Based on the ability of antioxidant to de-colorize the blue ABTS radical (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) by electron donation
- Express as μM Trolox equivalent/100 mg of dry extract

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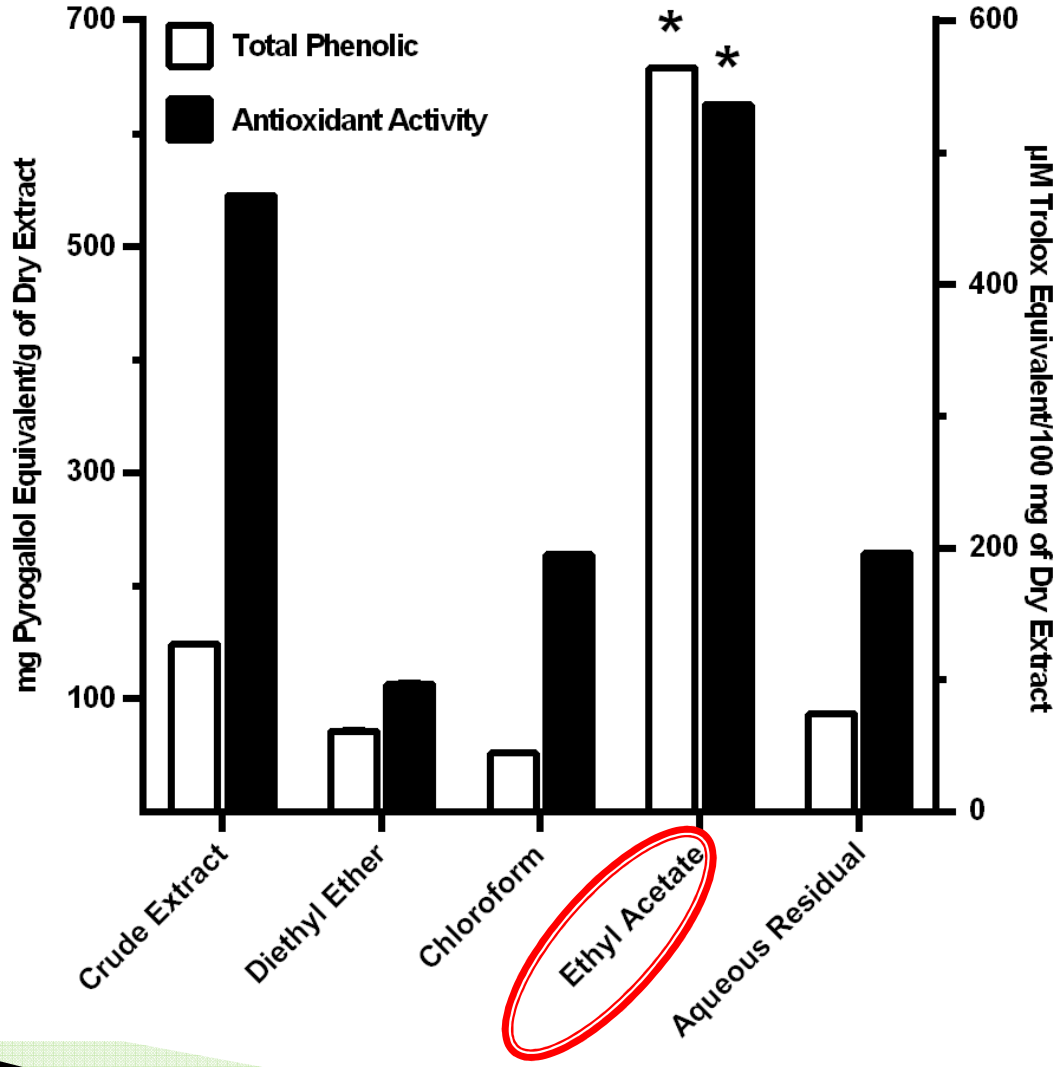
Result 1. Fractionation of the Phenolic and Antioxidant Activity of *Moringa oleifera Lam.*

The ethyl acetate fraction had the highest amounts of phenolic and oxidative activity compare to the other fractions.



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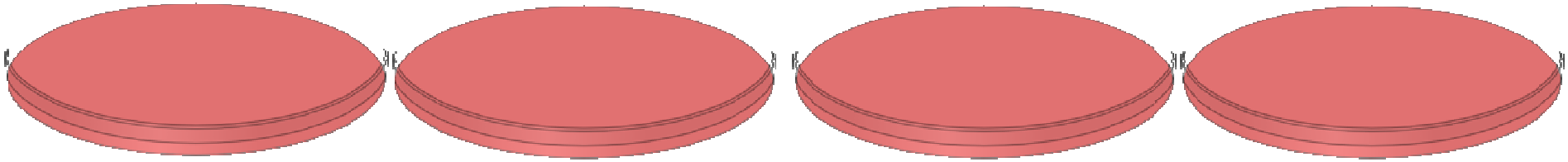
Determination of Cellular Cytotoxicity

Nicotine (NIC)

Cigarrete Smoke Extract (CSE)

Aspirin (ASA)

Ethyl Acetate of *Moringa Oleifera* Lam. (MOEF)



Cytotoxicity Determination

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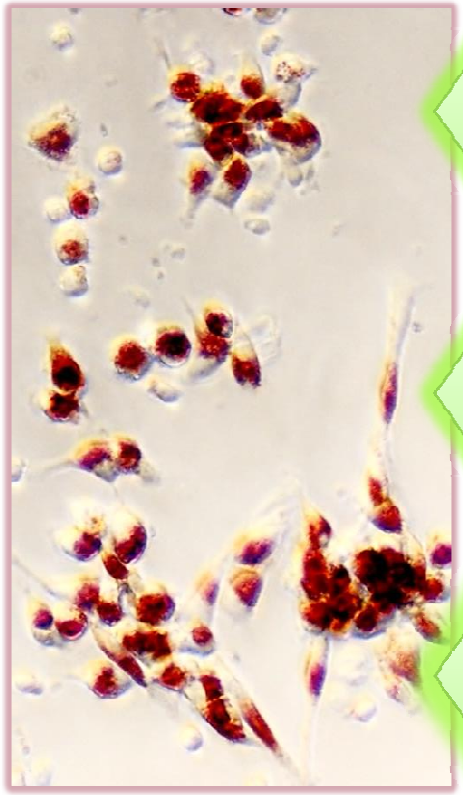
Determination of Cellular Cytotoxicity



The assay based on the ability of live cell to incorporate and bind to supra-vital dye, Neutral red.

The dye is positively charged, which accumulates in cellular cytoplasm and store in acidic condition of lysosome.

The amount of accumulated Neutral red is directly proportional to the amount of live cells in the cell culture.

Viability of cells were used to calculate the median lethal concentration of substances by Dose Response Sigmoidal Curve Fitting analysis.

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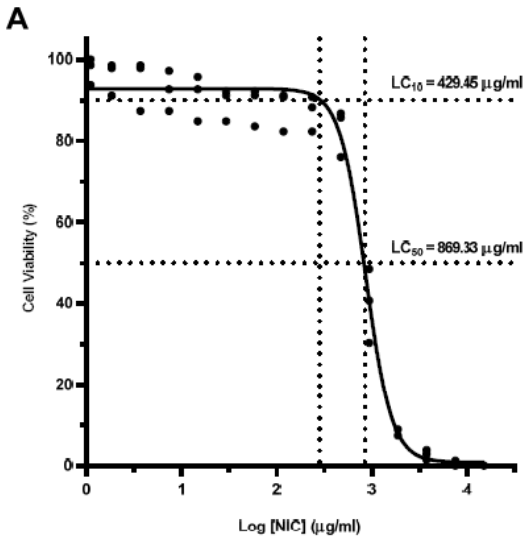
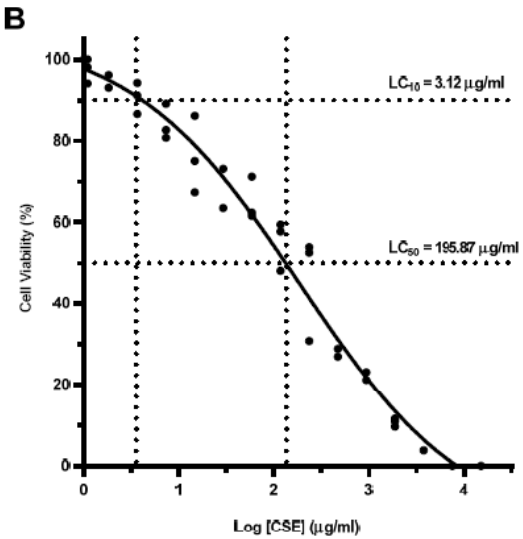
Result 2. Dose-response curves of each test substances showing LC_{50} and LC_{10}

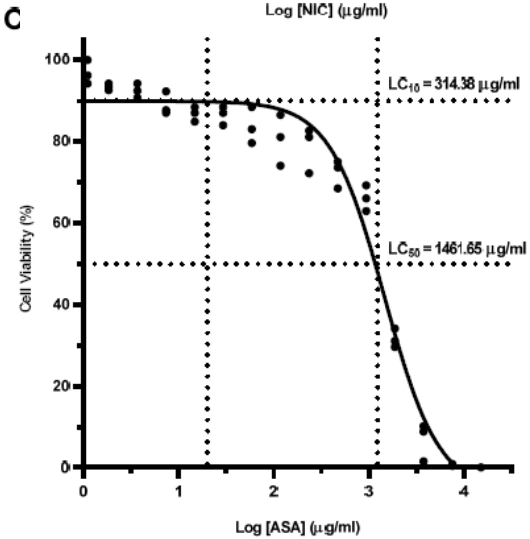
NIC

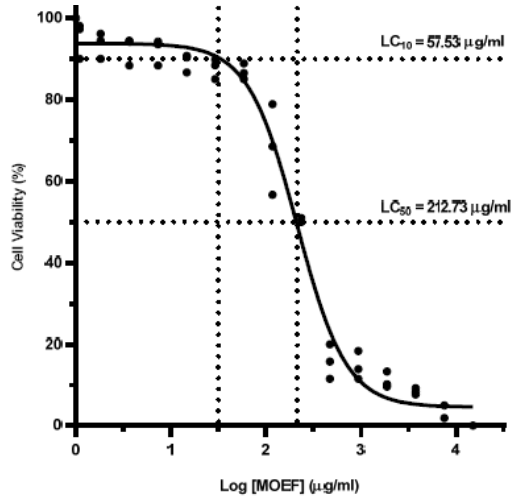




CSE

ASA





MOEF

LC_{10} had no effect on cell viability.

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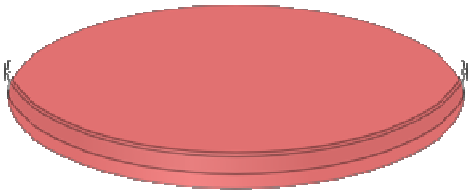
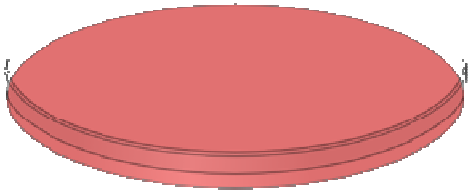
Induction of an Acute Inflammatory Response

10 ng/mL of LPS



2×10^5 of MDMs

33 ng/mL Nicotine equivalent of CSE



2×10^5 of MDMs

Real Time PCR
ELISA

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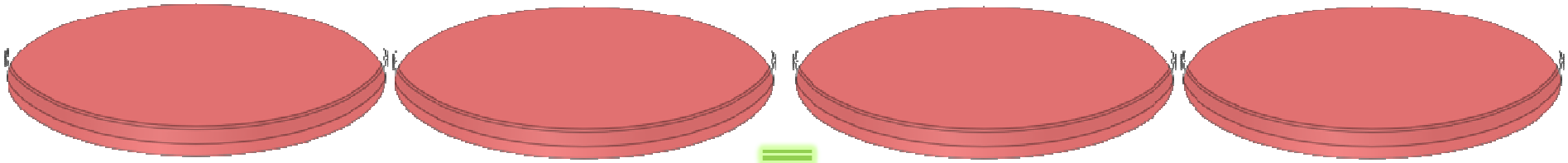
Investigation of Anti-Inflammatory Activity

341.38 µg/mL of ASA | 57.53 µg/mL of MOEF | 341.38 µg/mL of ASA | 57.53 µg/mL of MOEF



6 h

10 ng/mL of LPS | 10 ng/mL of LPS | 33 ng/mL Nicotine equivalent of CSE

12 h

Real Time PCR
ELISA

LPS; untreated control
Nicotine; control for CSE
ASA; positive control for an anti-inflammatory effect

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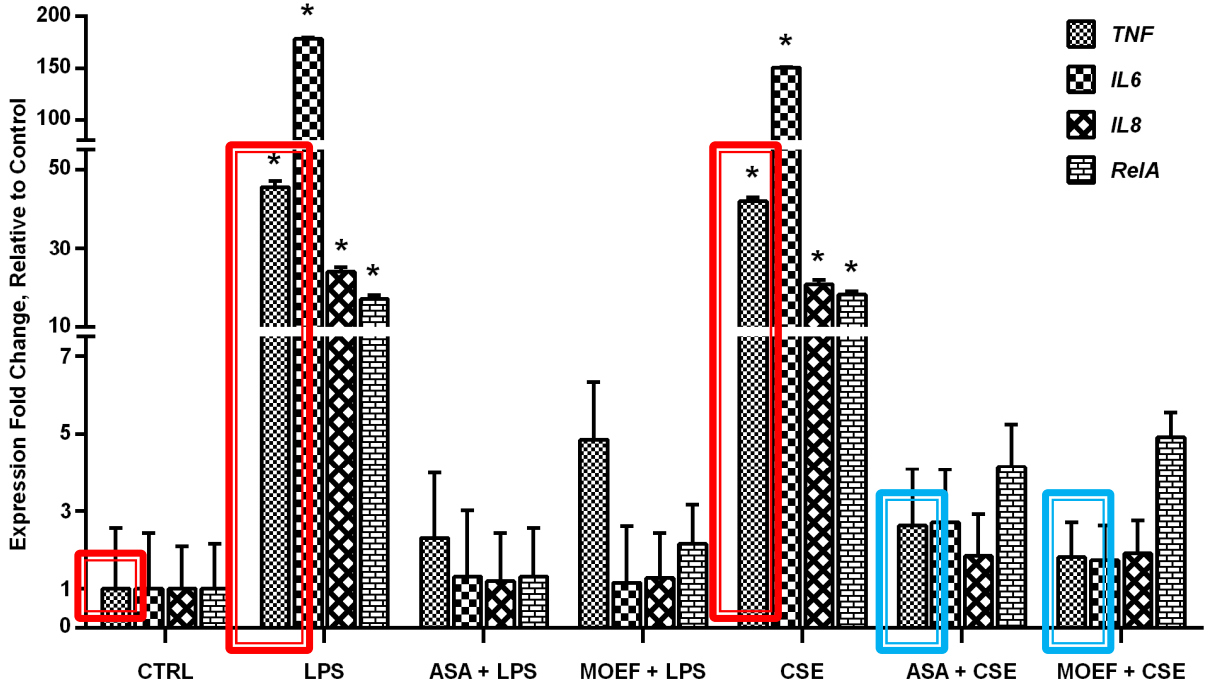
Result 3. Effects of MOEF on LPS- and CSE-induced *TNF, IL-6, IL-8 and RelA gene* expression in human MDM.

TNF from cell stimulated with either LPS or CSE were 40 fold relative to the non-stimulated cell.

ASA and MOEF cause almost complete inhibition of *TNF*.



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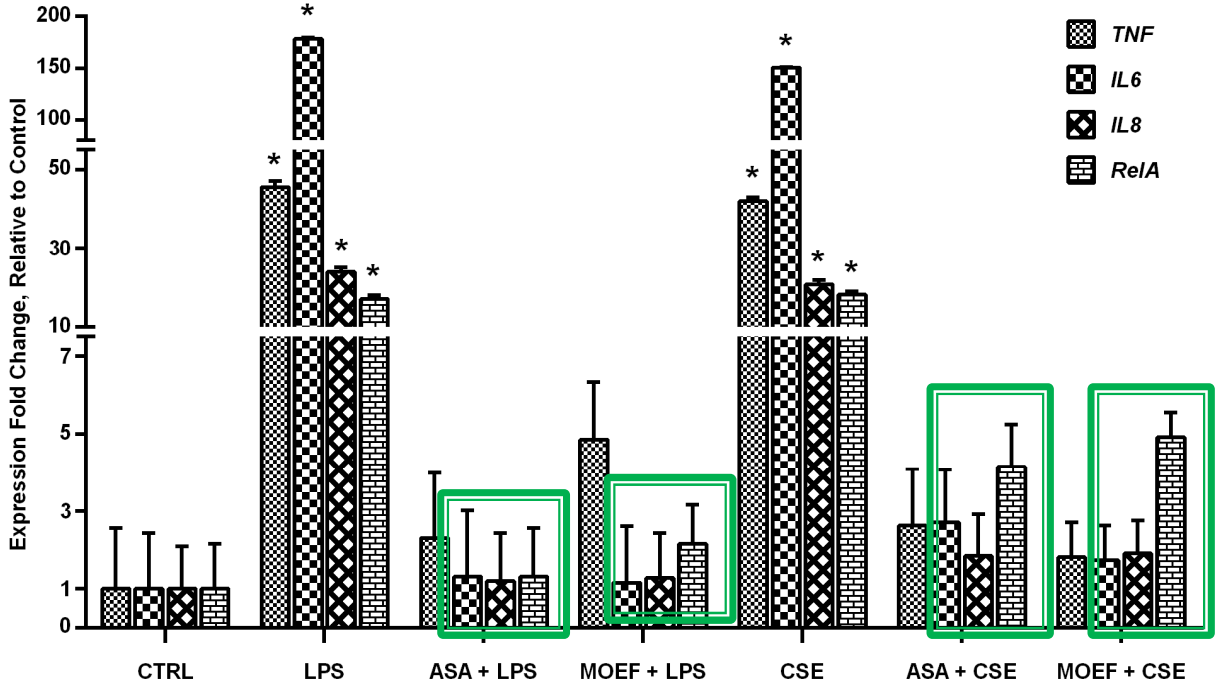
Result 3. Effects of MOEF on LPS- and CSE-induced *TNF, IL-6, IL-8 and RelA gene* expression in human MDM.

ASA and MOEF cause a similar inhibition of the LPS and CSE-induced *RelA, IL-6* and *IL-8* gene expression.



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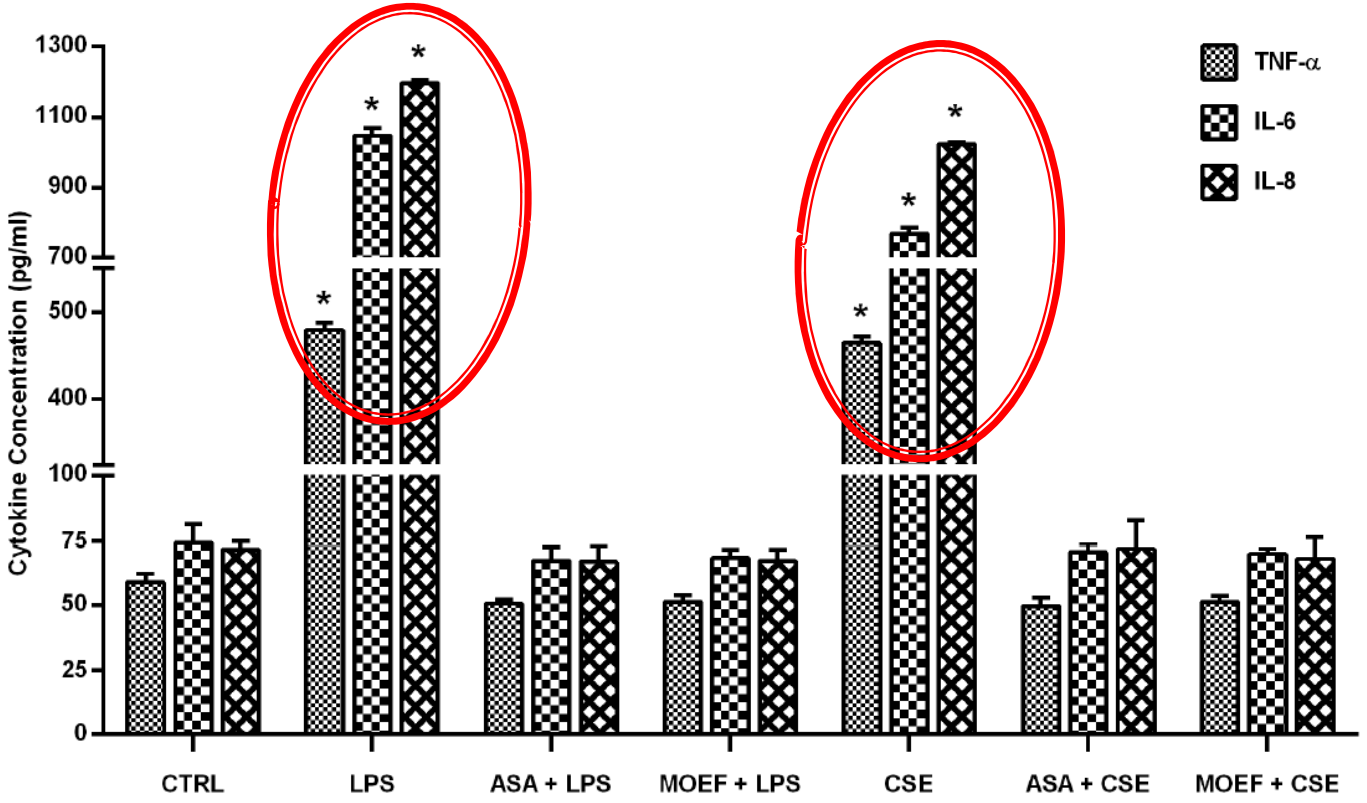
Result 4. Effects of MOEF on LPS- and CSE-induced TNF, IL-6 and IL-8 production by human MDM.

LPS and CSE caused approximately 10 fold increase in TNF, IL6 and IL-8.



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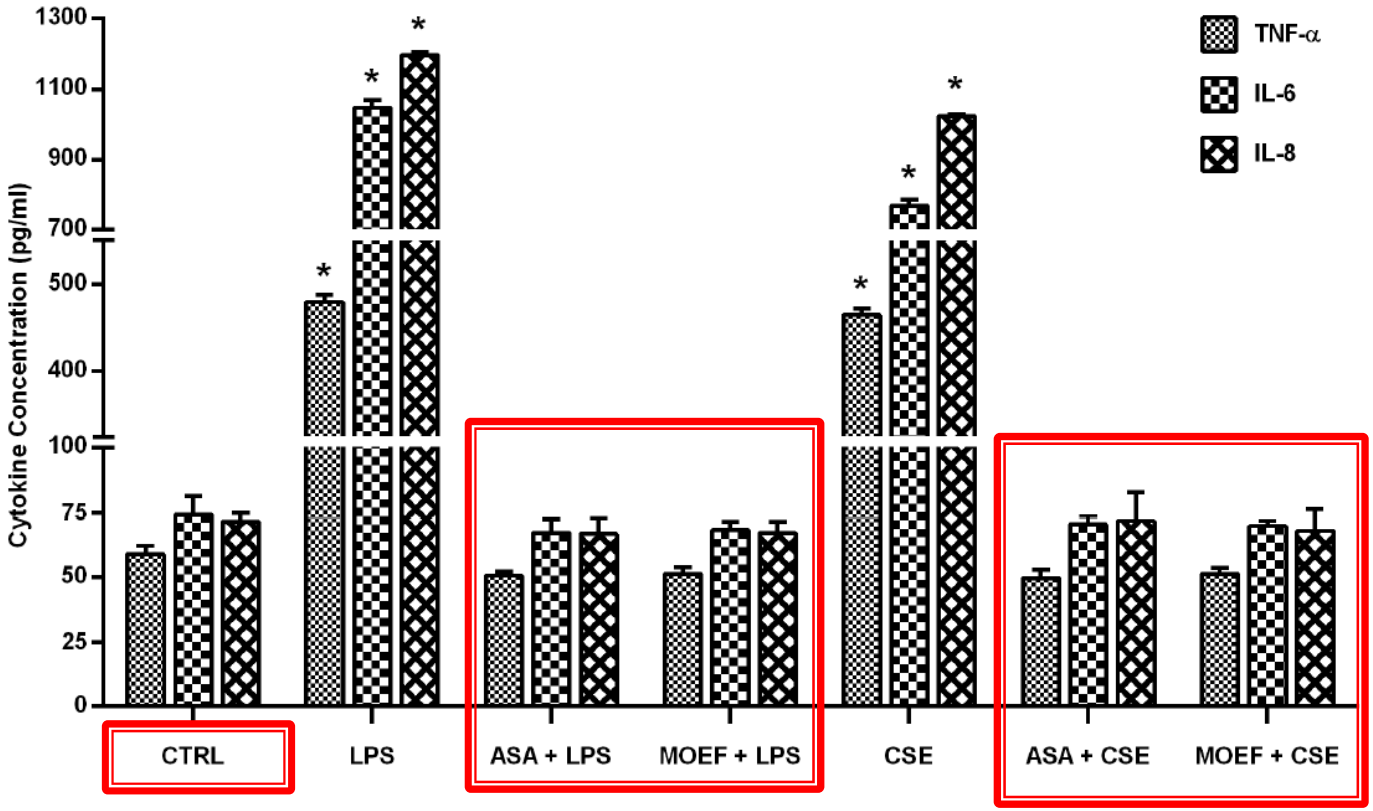
Result 4. Effects of MOEF on LPS- and CSE-induced TNF, IL-6 and IL-8 production by human MDM.

Both ASA and MOEF reduced LPS/CSE-induced cytokine production to basal level.



The result show that pretreated MOEF can decreased production of TNF, IL-6 and IL-8 in response to both LPS and CSE.

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Conclusions

- The mechanism of the anti-inflammatory effects of MO which may explain the beneficial effects of this plant in treating chronic inflammatory diseases.
- Phenolic rich fraction of MO inhibits cytokine production by human macrophages in an *in vitro* model of CSE-induced macrophage TNF, IL-6 and IL-8 production.
- The MOEF depress the expression of *RelA, a gene* important in NF-κB signaling inflammatory reaction.
- Similar results were found when LPS was used to stimulate these macrophage functions, suggesting an effect on a wider range of macrophage agonists.

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