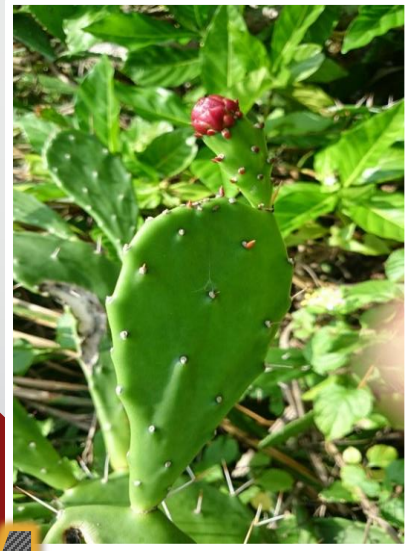
"Inhibition of Pro-inflammatory Mediators by Methanolic Extract of *Opuntia monacantha* Haw. (Cactaceae) in RAW 264.7 macrophages cells"



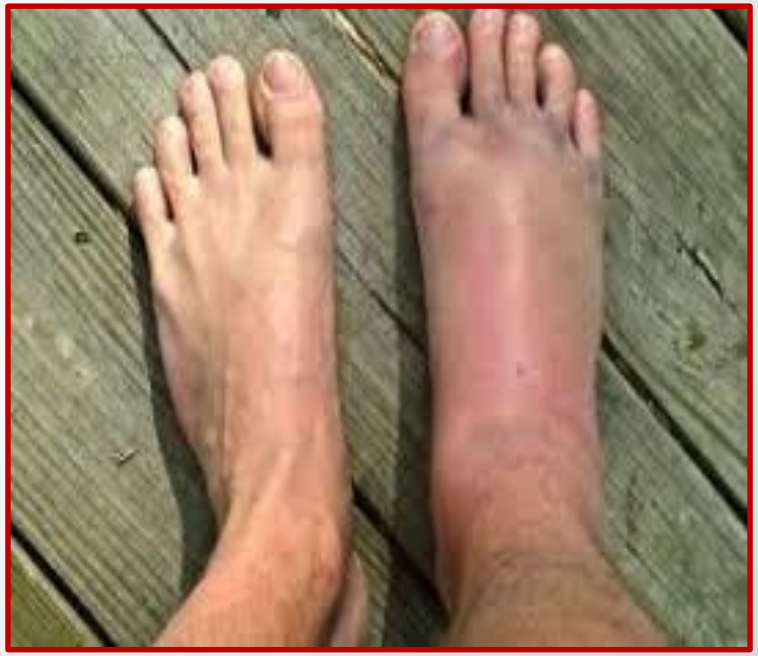
ERMAN SHAH JAIOS
PD09
Product Development Technology
FACULTY OF AGRO-BASED INDUSTRY (FIAT)
University Malaysia Kelantan (UMK)

Universiti Malaysia KELANTAN

4th POSTGRADUATE SYMPOSIUM FIAT

Virtual Symposium 2021

INTRODUCTION



Inflammation is the local response of living tissue to injury that involves a well-organized cascade of fluidic and cellular changes within living tissue.

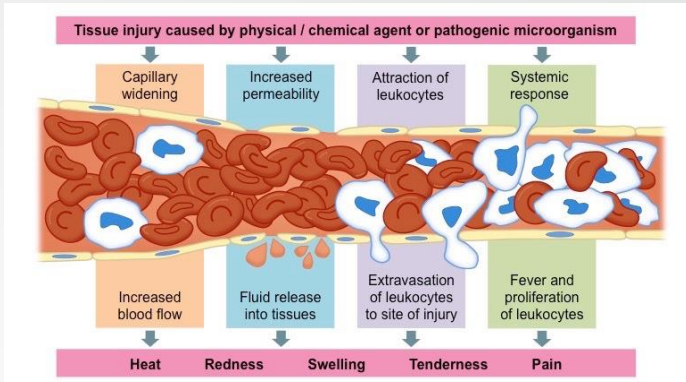
Body defense reaction - eliminate or limit the spread of initial cause of cell injury involving immune cells, blood vessels and molecular mediators.

Cause of inflammation:

1. **Infective agents**; bacteria, viruses, toxins, fungi, parasites.
2. **Immunological agents**; like cell-mediated and antigen antibody reaction.
3. **Physical agents**; like heat, cold, radiation, mechanical trauma.
4. **Chemical agents**; like organic and inorganic poisons.
5. **Inert materials**; such as foreign bodies.

- **Various medicines; to <u>control and suppress inflammation</u> e.g., steroids, non-steroid anti-inflammatory drugs, and immunosuppressant, associated with adverse effects** (Mona Ghasemian *et al.*, 2016)
- **Plant-based drugs used in the traditional medicine; great attention due it is easily available, higher efficacy, less expensive and less/no side effects** (Cathrine L *et al.*, 2011; Deepa Murugesan *et al.*, 2014; Yifu Yang *et al.*, 2015; ES Jaios *et al.*, 2016).

GENERAL SCHIMATIC DIAGRAM OF INFLAMMATORY PROCESS



Harmful/hazardous stimuli
endogenous/exogenous inducer of inflammation
e.g; microbial / non-microbial

'response/trigger'
(production of numerous inflammatory mediators by cells or plasma protein)

- *Cytokines* e.g., TNF-α produced by macrophage (cell types)
- iNOs → NO
- *Lipid mediators;*
  AA → *metabolized by* COX_1 & COX_2 → PGE_1 & PGE_2

'Inflammatory response'
(alter functioning of tissue and organ)

at affected area of tissue injury /damaged tissue

- Release inflammatory cytokines/mediators
- Dilation and leakier of blood vessels
- Attraction of WBC
- Fluid secretion

'ACUTE INFLAMMATION/induction of acute-phase response

Hyper-algesic & fever by PGE_2

Redness (rubor)
Swelling (tumor)
Heat (colour)
Pain (dolor)

Triggering pain receptors *(pain & loss of function)*

'IMMUNE RESPONSE' / Healing & Repair

- Repair & Restoration (regeneration and replacement) of lost cells e.g., in bone, nervous tissue and myocardium
- Vascular response (granulation tissue by proliferation fibrous tissue e.g., wound construction) (granulomatous/inflammatory infiltration by macrophages)
- Maturation; normal structure & function of tissue

'CHRONIC INFLAMMATION'
Prolonged inflammation/ acute symptoms fails to heal injury

Vascular damage
Tissue Hypoxia / degeneration

Opuntia monacantha & Pharmacological Activities

Family : Cactaceae , genus : *Opuntia*

- ✓ Prickly pears typically grow with flat, rounded cladodes 'platyclades'.

Distribution: (Griffith MP, 2004; Saenz C, 2000; Inglese P *et al.,* 2002)

- According to previous reports, family Cactaceae is contain about 130 genera nearly 1500 all well adapted to arid lands, to a diversity of climates and are naturalized in several areas all over the world, including the Mediterranean, Middle East, South Africa, Australia and India.
- In South Africa, Mediterranean and South American; some species is also cultivated for its edible fruit (prickly pear), although in some countries different parts of the plant are utilized in the food and cosmetic industry.

✓ revealed phytochemical constituents of alkaloids, flavonoids, tannins and saponins (MN Bari *et al*., 2012).

◉ Traditional uses and pharmacological properties of *Opuntia* sp.

Common names	Cultural uses	Pharmacological uses
Indiang fig Barbary fig Tuna cactus Cactus pear Nopal Mission cactus Smooth mountain Prickly pear	- Treatment of diabetes, - hypertension, - hypolipidemic, asma, ulcers, rheumatic pain, wounds, and fatigue De Smet PA, 2002 Pareek OP *et al.*, 2003 Saenz C, 2000 Inglese P *et al.*, 2002	Anti-diabetic (Zhao LY *et al.*, 2011; Hahm SW *et al.*, 2011) Anti-hypercholesterolemic (Oh PS *et al.*, 2006) Anti-hyperlipidemic (Oh PS *et al.*, 2006) Anti-stress (Perfumi M *et al.*, 1996) Anti-uric and diuretic (Park EH *et al.*, 2001) Anti-inflammatory (Palevitch D *et al.*, 1993) Anti-cancer (Wie MB *et al.*, 2000) Neuro-protector (Kim JM *et al.*, 2010; 2006)

OBJECTIVES

GENERAL OBJECTIVE

- To evaluate the anti-inflammatory activities of methanolic extract of *Opuntia monacantha Haw.* (MEOM) and its mechanisms of action using *in vitro* models of inflammation.

SPECIFIC OBJECTIVES

1. To determine effect of MEOM on NO production in the RAW 264.7 macrophages induced by LPS/IFN-γ using Griess assay.
2. To determine cytotoxicity effect of MEOM on cell viability in the RAW 264.7 macrophages induced by LPS/IFN-γ using MTT assay.
3. To elucidate the possible mechanism of action that takes part in the anti-inflammatory of MEOM on pro-inflammatory mediators/cytokines production in the RAW 264.7 macrophages induced by LPS/IFN-γ using sandwich ELISA.
4. To identify the phytoconstituents profiling that present in the MEOM using UHPLC-Q-TOF/MS.

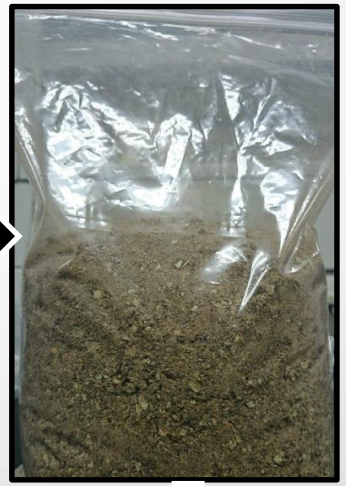
RESEARCH METHODOLOGY

SAMPLING & AREA COLLECTION

- Location of sampling (*Opuntia monacantha*): coastal area in Tok Bali, Kelantan, Malaysia
- Identified by the Institute of Bioscience (IBS), deposited at UPM from the Herbarium of IBS, UPM, Malaysia
- Voucher no: SK 2881/15

Natural habitat
- Collection &
- Identification

cladodes shaded/air-dried for 1 – 2 weeks, RT (27 ± 2°C)



Dried clododes-grind (small particles)

PREPARATION OF THE METHANOLIC EXTRACT (MEOM)

Soak (absolute MeOH) at RT for 72h x 3 times, ratio of 1:20 (w/v)

filter & collect methanol supernatant by sintered glass filter with vacuum pump

methanol extract – evaporation process (40°C) under reduced pressure to dryness & obtain the crude dried extracts.

Setting for evaporation at 40°C.
Vacuum: 337mbar (for methanol b.p 65°C)

Zakaria et al. 2007b; ES Jaios et al., 2016 with slight modifications.

RESEARCH METHODOLOGY

Inhibitory of the Pro-inflammatory Mediators by MEOM

1. Assessment on Cytotoxicity (cell viability) of MEOM

The cell viability and cytotoxicity activity of sample extract was determined using **MTT** (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay (Achoui M *et al*., 2010; Zakaria *et al*., 2015, with slight modifications) & **DMSO**-treated group as negative control (C).

2. Assessment on LPS/IFN-γ-induced NO production

The NO production was determined using the **Griess reaction** (Zakaria *et al*., 2015, with slight modifications). The amount of nitrite (a stable metabolite of NO), was used as the indicator of NO production. **L-NAME**, a standard iNOS inhibitor (acts as an inhibitor of NO synthase) as positive control & **DMSO**-treated as negative control (C).

3. Assessment on LPS/IFN-γ-induced PGE_2, iNOs, COX-2, TNF-α and LOX production

The level of **PGE_2** (Yang *et al*., 2012), **iNOs** & **COX-2** (Chu-Wen Li *et al*., 2013; Gao et al., 2015), **TNF-α** (Xiaojuan Xu *et al*., 2011; Chu-Wen Li *et al.,* 2013; Katyakyini Muniandy *et al*., 2018), and **LOX** (Rekha Bisht *et al*., 2014; Noor Wahida Ismail Suhaimy *et al*., 2017) in the supernatants were measured using ELISA according to the manufacturer's instructions, and method described in detailed with slight modifications.

Phytoconstituents profiling UHPLC-Q-TOF/MS Acquisition Analysis

The analysis method and mass detection of synthetic compounds of MEOM was done by LCMS Unit (1290 UHPLC and 6520 Q-TOF mass spectrometer, Agilent Corp, USA), iPROMISE, UiTM, Selangor, Malaysia.

STATISTICAL ANALYSIS

The one-way ANOVA-Dunetts's post hoc tests were used to determine the statistical significance of differences between the values for the various experimental and control. Data are expressed as means ± S.E.M (at least three independent experiments performed in triplicate). *P*-Values of 0.05 or less were considered statistically significant.

RESULTS & DISCUSSION

Assessment on LPS/IFN-γ-induced inflammation on Cytotoxicity (Cells Viability)

method described in detailed by Yang *et al*., 2012; Zakaria *et al*., 2015, with slight modifications.

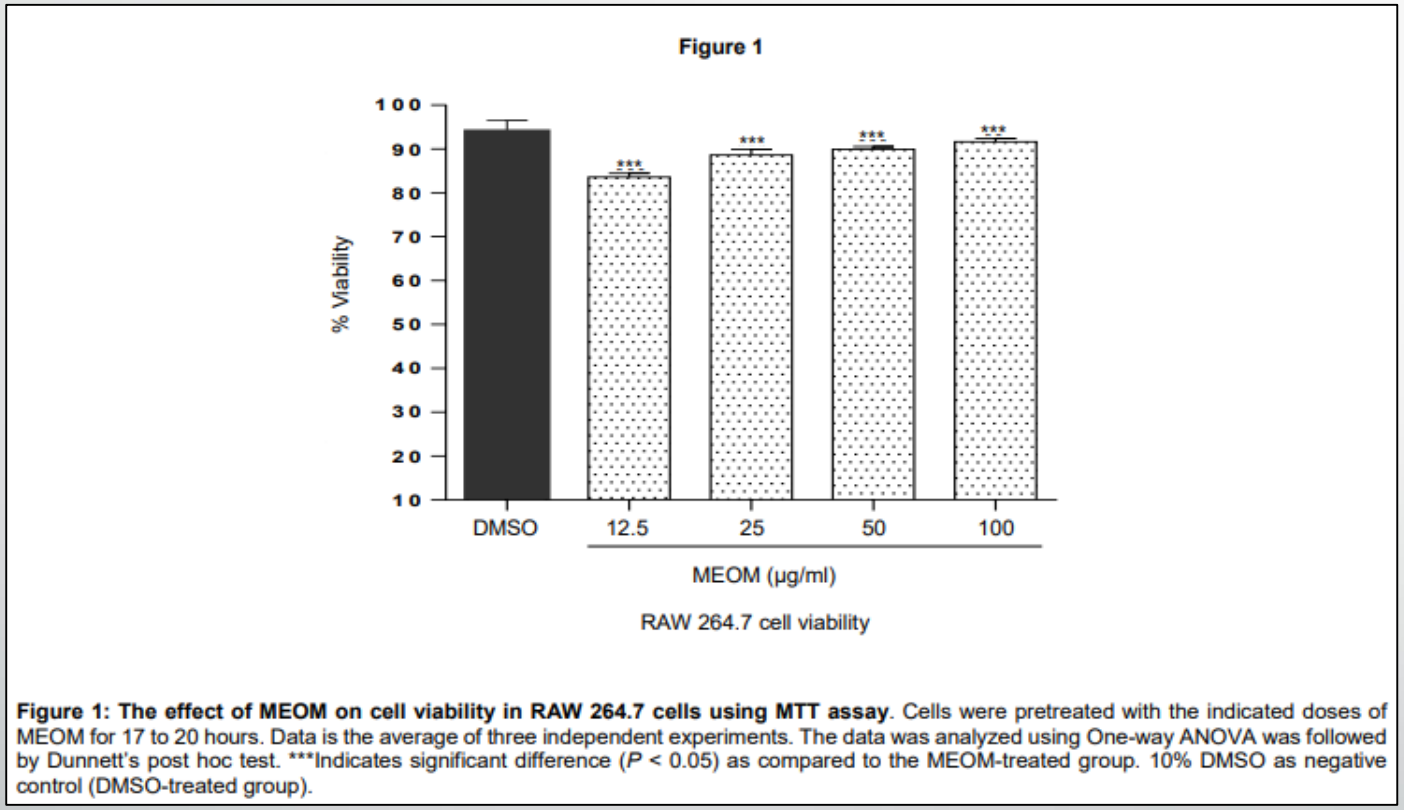


Figure 1: The effect of MEOM on cell viability in RAW 264.7 cells using MTT assay. Cells were pretreated with the indicated doses of MEOM for 17 to 20 hours. Data is the average of three independent experiments. The data was analyzed using One-way ANOVA was followed by Dunnett's post hoc test. ***Indicates significant difference ($P < 0.05$) as compared to the MEOM-treated group. 10% DMSO as negative control (DMSO-treated group).

The effect of MEOM on cell viability was evaluated using the MTT colorimetric assay. As depicted in Figure 1, following a 17 to 20 h treatment, MEOM had no effect on RAW 264.7 cell viability (or more than 80% towards cells growth) with the concentrations ranging from 12.5-100 µg/mL.

RESULTS & DISCUSSION

Assessment on LPS/IFN-γ-induced inflammation on NO production (NO determination)

method described in detailed by Yang *et al*., 2012; Zakaria *et al*., 2015, with slight modifications.

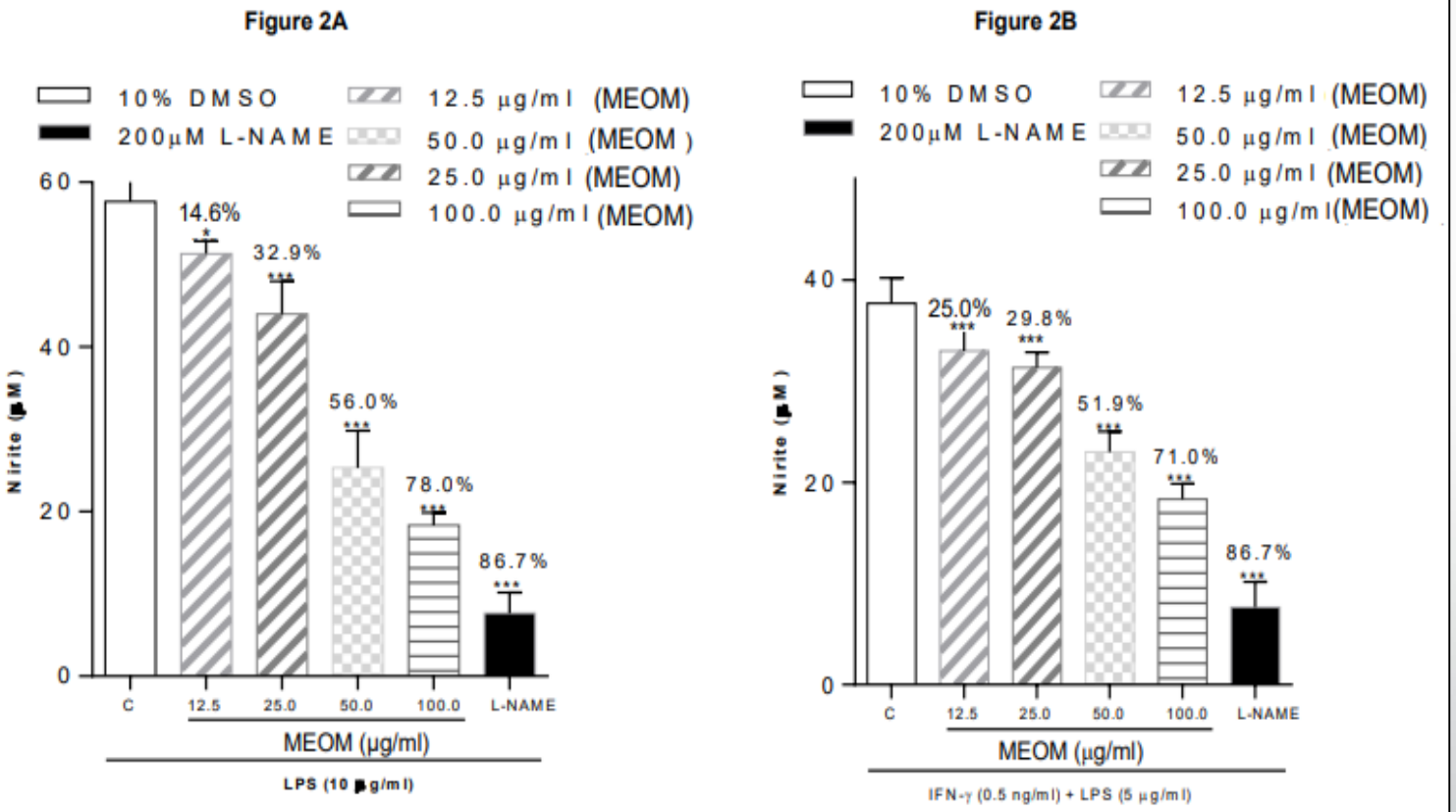


Figure 2: The effect of MEOM on LPS/IFN-γ-induced NO levels in RAW 264.7 cells. (A) Cells were treated with different concentrations of MEOM (12.5, 25, 50 and 100 µg/ml) for 1 h, then with LPS (10 µg/ml) for 24 h, and analyzed for nitrite levels. (B) Nitrite levels was measured after the cells were primed with IFN-γ (0.5 ng/ml) for 2 h and then stimulated with LPS (5 µg/ml) in the presence of MEOM for 18 h. The inhibition concentration was measured using ELISA. Data represents a means of three replicates with mean ± S.E.M and analysed using one-way ANOVA was followed by Dunnett's post hoc test. ***Data differed significantly ($P < 0.05$) when compared to the 10% DMSO-treated group. *Data differed not significant as compared to the 10% DMSO-treated group.

Cells; pre-incubated with concentrations of MEOM (12.5, 25, 50 and 100µg/mL) for 1 hr, and than,

- ✓ Figure 2A: stimulated with 10 µg/mL LPS alone for 24 h, and
- ✓ Figure 2B: pre-treated with IFN-γ (0.5 ng/mL) for 2 h, and then stimulated with LPS (5 µg/mL) for 18 h.

MEOM; indicates to increased, significantly ($P < 0.05$) the percentage of inhibition of NO production between:

- ✓ Figure 2A: 32% and 78%, and
- ✓ Figure 2B: 29% and 71%

MEOM; not added to the –ve controls (C), DMSO
L-NAME, iNOS inhibitor; exhibited highest significantly ($P < 0.05$) inhibition of NO production in the cells.

- ✓ The nitrite level in the cell was determined, and observed that MEOM reduced NO concentration in a dose-dependent manner, respectively.

RESULTS & DISCUSSION

Assessment on LPS/IFN-γ-induced inflammation on Prostaglandin (PGE_2) Production

method described in detailed by Yang *et al*., 2012; Zakaria *et al*., 2015, with slight modifications.

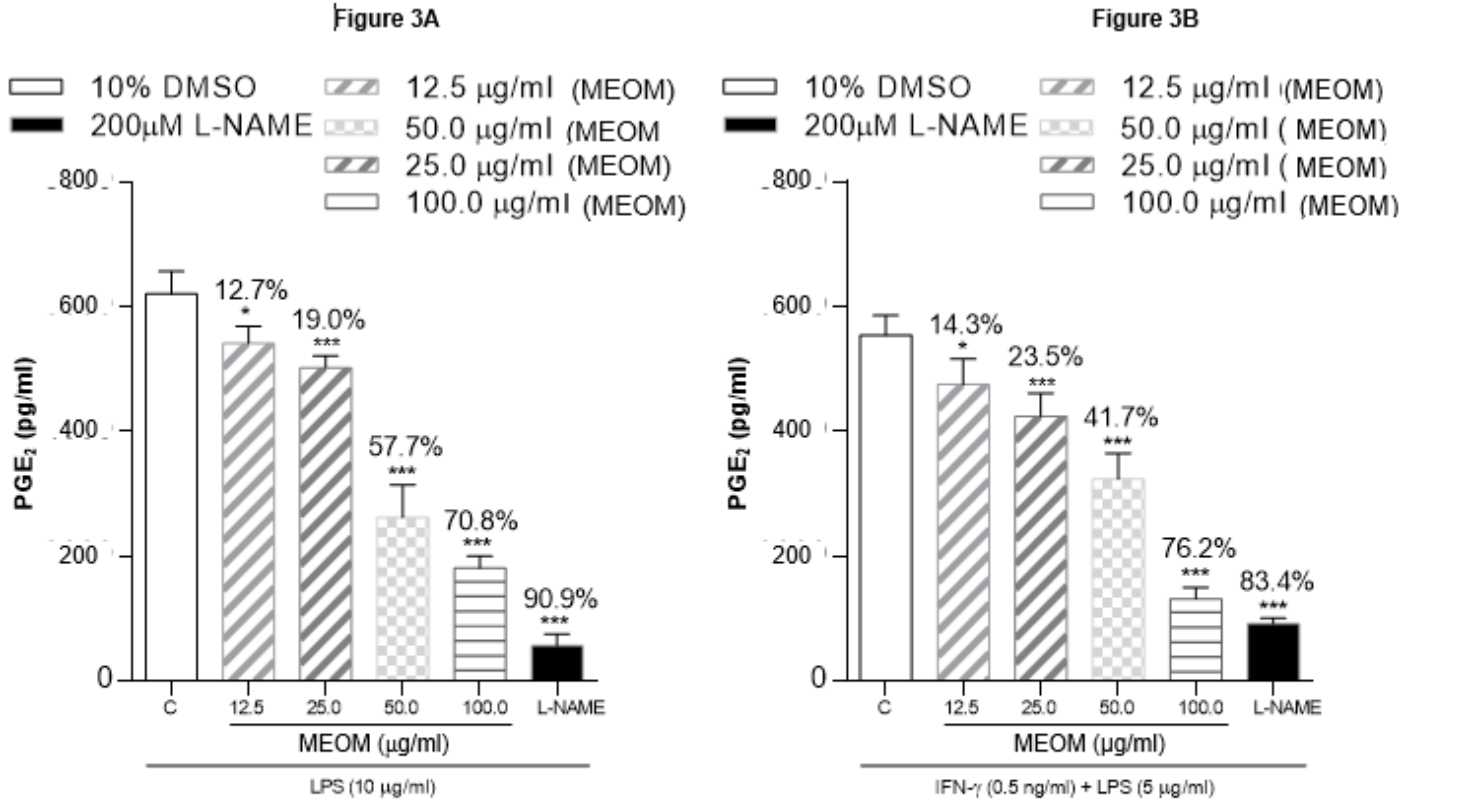


Figure 3: The effect of MEOM on LPS/IFN-γ-induced PGE_2 production in RAW 264.7 cells. (A) PGE_2 production was measured in RAW 264.7 Cells were treated with different concentrations of MEOM (12.5, 25, 50 and 100 μg/ml), then incubated with LPS (10 μg/ml) for 24 h. (B) PGE_2 production was measured after were primed with IFN-γ (0.5 ng/ml) for 2 h and then stimulated with LPS (5 μg/ml) in the presence of MEOP for 18 h. The inhibition concentration was measured using ELISA. Data represents a means of three replicates with mean ± S.E.M and analysed using one-way ANOVA was followed by Dunnett's post hoc test. ***Data differed significantly ($P < 0.05$) when compared to the 10% DMSO-treated group. *Data differed not significant as compared to the 10% DMSO-treated group.

Cells; produce PGE_2 when stimulated by LPS and/or IFN-γ.

The PGE_2 level in the cell was determined, and observed that MEOM reduced PGE_2 production in a dose-dependent manner (figure 3A & 3B).

The extract; indicates to increased, significantly ($P < 0.05$) the percentage of inhibition of PGE_2 production between:

- ✓ Figure 3A: 19% and 70%, and
- ✓ Figure 3B: 23% and 76%.

MEOM were not added to the –ve controls (C), DMSO
Level of PGE_2 production was reduced significantly ($P < 0.05$) by L-NAME, an inhibitor of PGE_2 production, which was highest percentage of inhibition.

The level of PGE_2 production in the cell was determined, and observed that MEOM reduced PGE_2 concentration in a dose-dependent manner,
respectively

RESULTS & DISCUSSION

Assessment on LPS/IFN-γ-induced inflammation on Cyclooxygenase-2 (COX-2) Production

method described in detailed by Chu-Wen Li *et al*., 2013; Zakaria *et al*., 2015, with slight modifications.

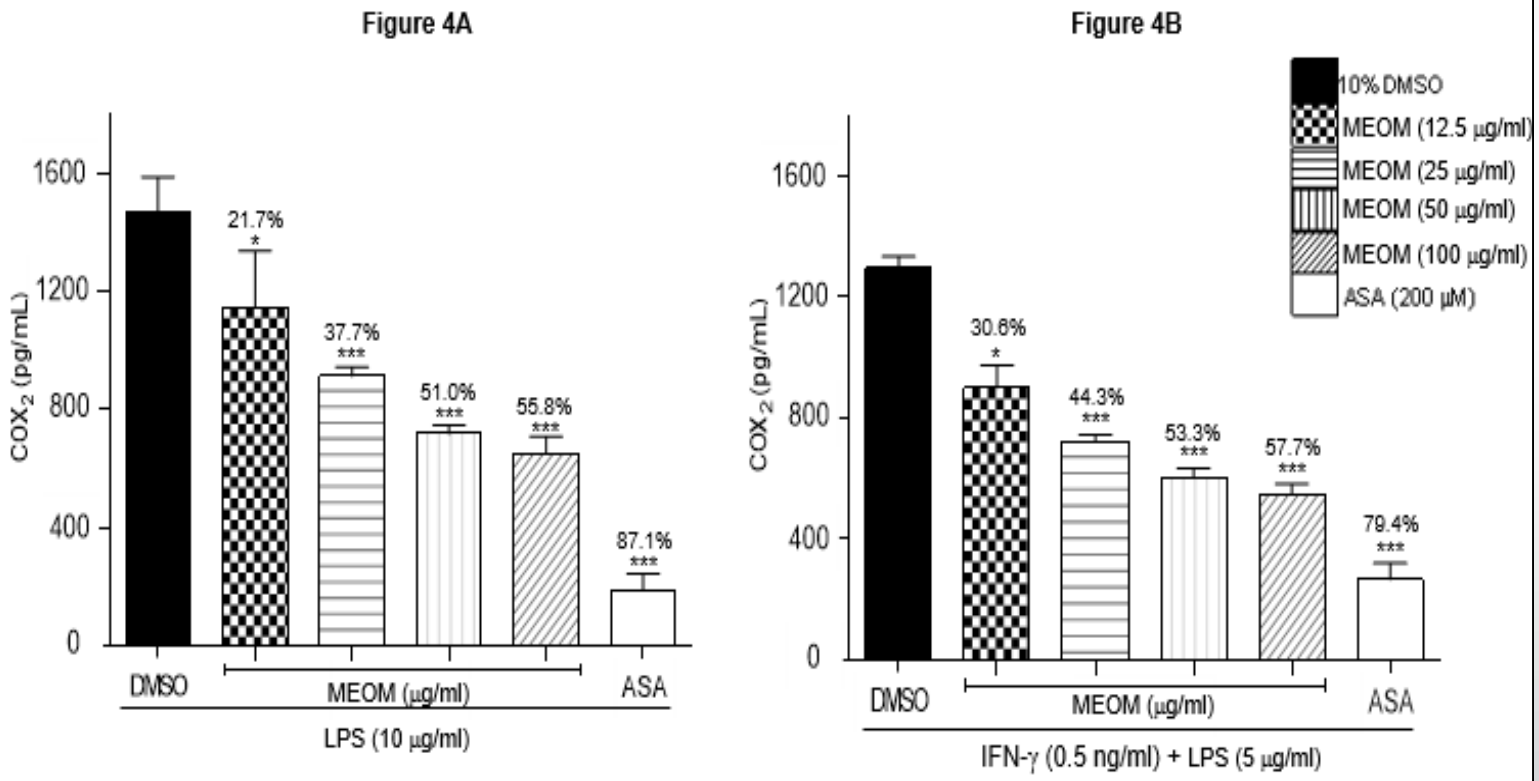


Figure 4: Effect of MEOM on LPS/IFN-γ-induced COX-2 production in RAW 264.7 cells. (A) COX-2 production was measured in RAW 264.7 Cells were treated with different concentrations of MEOM (12.5, 25, 50 and 100 µg/ml), then incubated with LPS (10 µg/ml) for 24 h. (B) COX-2 production was measured after were primed with IFN-γ (0.5 ng/ml) for 2 h and then stimulated with LPS (5 µg/ml) in the presence of MEOM for 18 h. The inhibition concentration was measured using ELISA. Data represents a means of three replicates with mean ± S.E.M and analysed using one-way ANOVA was followed by Dunnett's post hoc test. ***Data differed significantly ($P < 0.05$) when compared to the 10% DMSO-treated group. *Data differed not significant as compared to the 10% DMSO-treated group.

Effects of MEOM on the COX-2 production level were shown in Figures 4 (A & B).

Stimulation of LPS/IFN-γ on RAW 246.7 cells line induced production the NO-inflammation and increased the level of COX-2 in the cultured cells.

Treatment of MEOM in the ranging between 12.5 – 100 µg/ml, significantly decreased the production of COX-2-dose dependently ($P < 0.05$ versus control-treated group), between:

- ✓ Figure 4A: 37% and 55%, and
- ✓ Figure 4B: 44% and 57%

MEOM were not added to the –ve controls (C), DMSO
Selective anti-inflammatory drugs such as Acetylsalicylic acid (ASA) significantly, attenuated the production of COX-2 as a pro-inflammatory mediator, which considered major agents to produce inflammation of macrophages, respectively.

RESULTS & DISCUSSION

Assessment on LPS/IFN-γ-induced inflammation on Inducible Nitric Oxide synthase (iNOs) Production

method described in detailed by Chu-Wen Li *et al*., 2013; Gao *et al*., 2015, with slight modifications.

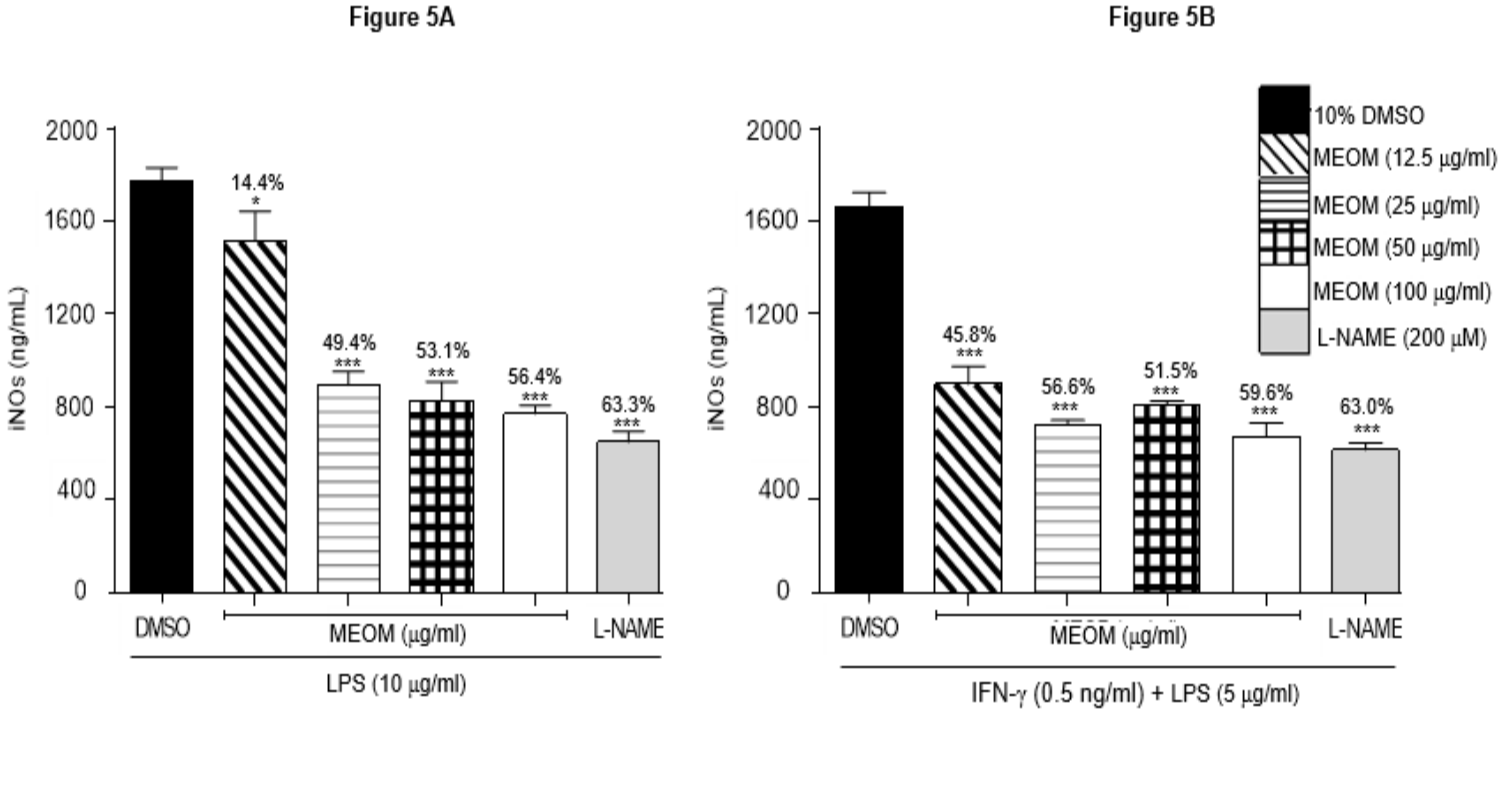


Figure 5: The effect of MEOM on LPS/IFN-γ-induced iNOs production in RAW 264.7 cells. (A) iNOs production was measured in RAW 264.7 Cells were treated with different concentrations of MEOM (12.5, 25, 50 and 100 µg/ml), then incubated with LPS (10 µg/ml) for 24 h. (B) iNOs production was measured after were primed with IFN-γ (0.5 ng/ml) for 2 h and then stimulated with LPS (5 µg/ml) in the presence of MEOM for 18 h. The inhibition concentration was measured using ELISA. Data represents a means of three replicates with mean ± S.E.M and analysed using one-way ANOVA was followed by Dunnett's post hoc test. ***Data differed significantly ($P < 0.05$) when compared to the 10% DMSO-treated group. *Data differed not significant as compared to the 10% DMSO-treated group.

The effects of MEOM on the iNOs production level were shown in figure 5 (A & B).

Stimulation of LPS/IFN-γ on RAW 246.7 cells line induced production the NO-inflammation and increased iNOs level in the cultured cells.

Treatment of MEOM in the ranging between 12.5 – 100 µg/ml, significantly decreased the production of iNOs-dose dependently ($P < 0.05$ versus control-treated group), between:

- ✓ Figure 5A: 49% and 56%, and
- ✓ Figure 5B: 45% and 59%.

MEOM were not added to the –ve controls (C), DMSO.
Selective anti-inflammatory drugs such as L-NAME (NO inhibitor) significantly, attenuated the production of iNOs as a pro-inflammatory mediators is considered major selective enzyme to produce iNOs mediated-NO-inflammation of macrophages, respectively.

RESULTS & DISCUSSION

Assessment on LPS/IFN-γ-induced inflammation on Lipooxygenase (LOX) Production

method described in detailed by Rekha Bisht *et al.,* 2014, with slight modifications.

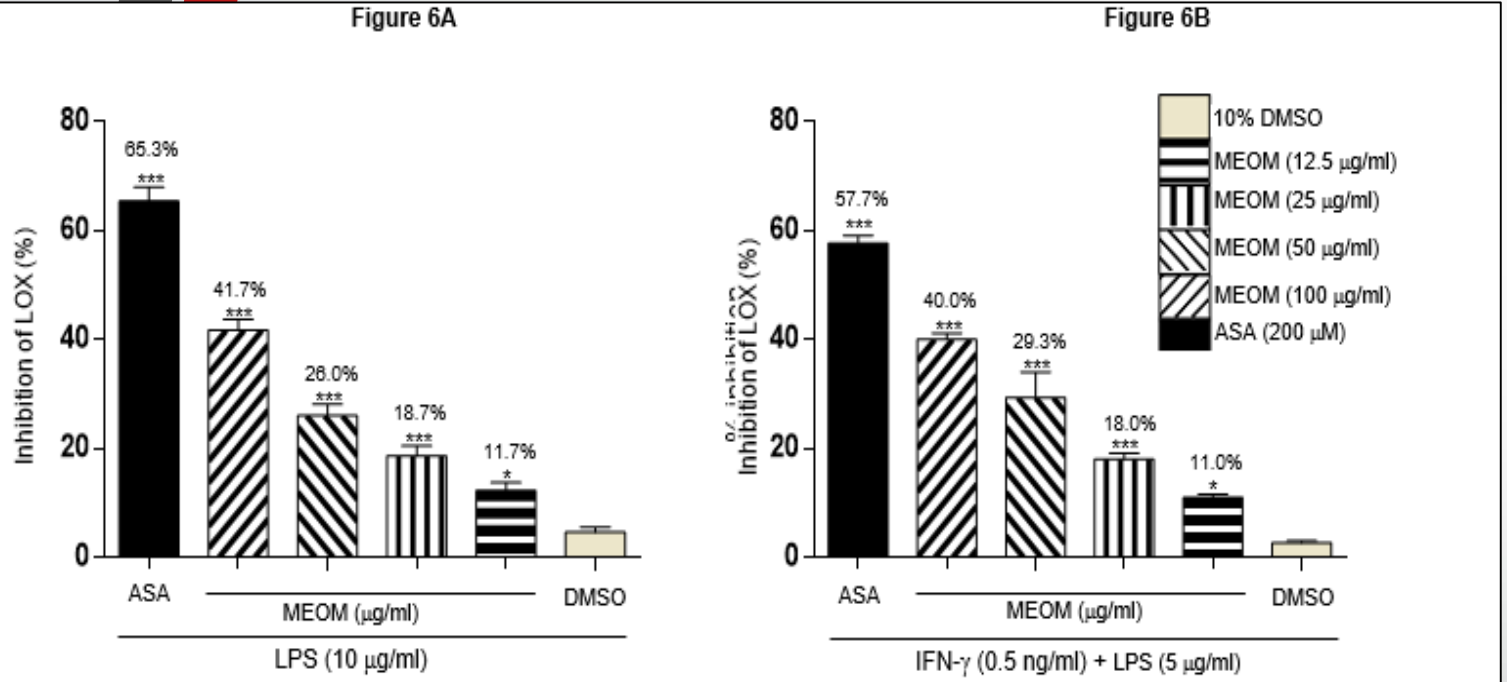


Figure 6: The effect of MEOM on LPS/IFN-γ-induced LOX production in RAW 264.7 cells. (A) LOX production was measured in RAW 264.7 Cells were treated with different concentrations of MEOM (12.5, 25, 50 and 100 µg/ml), then incubated with LPS (10 µg/ml) for 24 h. (B) LOX production was measured after were primed with IFN-γ (0.5 ng/ml) for 2 h and then stimulated with LPS (5 µg/ml) in the presence of MEOM for 18 h. Data represents a means of three replicates with mean ± S.E.M and analysed using one-way ANOVA was followed by Dunnett's post hoc test.
***Data differed significantly ($P < 0.05$) when compared to the 10% DMSO-treated group.
*Data differed not significant as compared to the 10% DMSO-treated group.

From the result obtained, maximum LOX inhibitory effect of MEOM was found to be 41.7% (figure 6A) and 40.0% (figure 6B) at dose, 100 µg/ml).

Pro-inflammatory mediator; LOX that induced by LPS/IFN-γ was exhibited by MEOM in dose dependent inhibition at the different concentration (12.5, 25 and 50 µg/ml), significantly between:

- ✓ Figure 6A: 41% and 18%, and
- ✓ Figure 6B, 40% and 18%

MEOM were not added to the –ve controls (C), DMSO

Overall result obtained, ASA at dose 200 µM, as the reference drug was exhibited the highest LOX inhibitory effect, respectively.

RESULTS & DISCUSSION

Assessment on LPS/IFN-γ-induced inflammation on Tumor Necrosis Factor-alpha (TNF-α) Production

method described in detailed by Chu-Wen Li *et al*., 2013, with slight modifications.

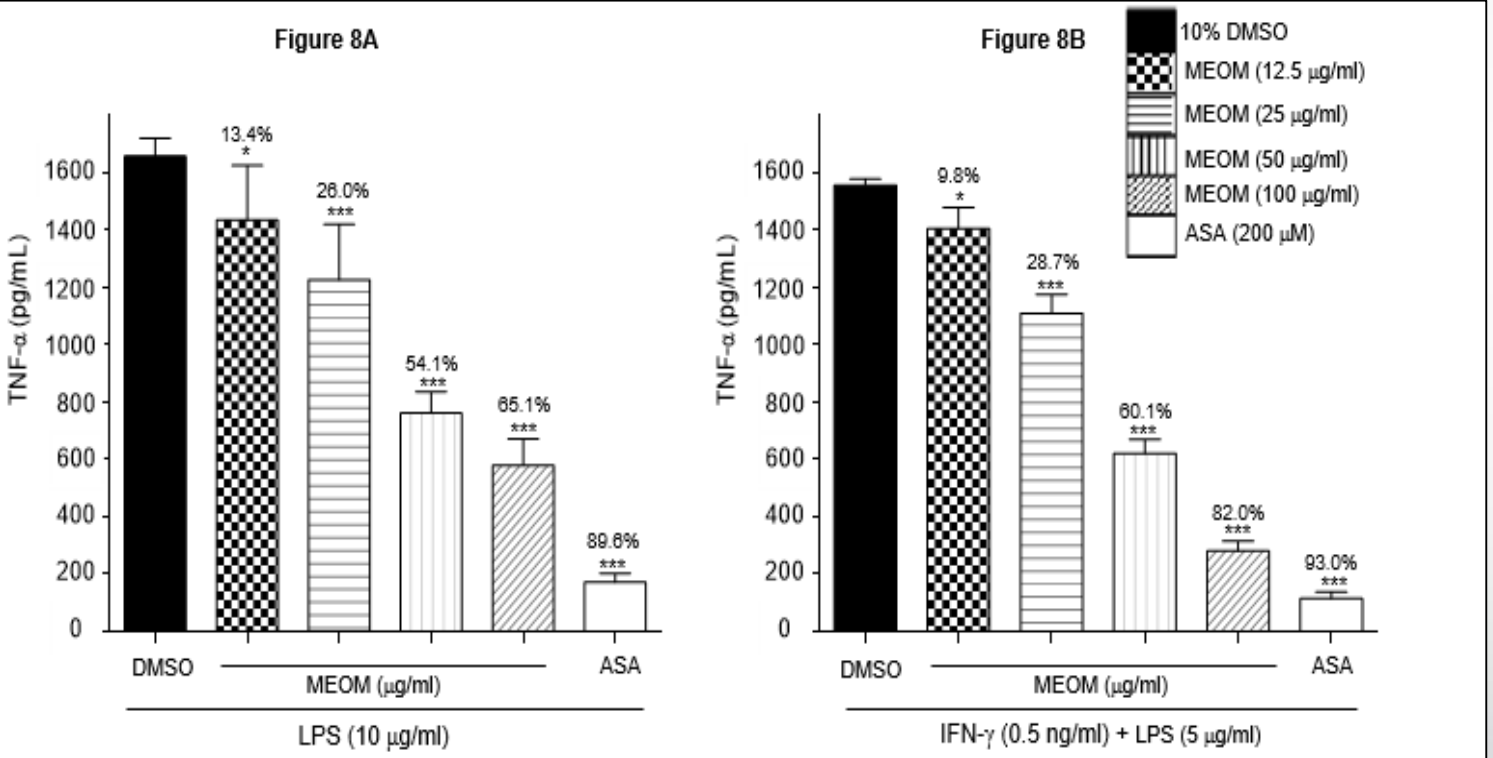


Figure 8: The effect of MEOM on LPS/IFN-γ-induced TNF-α production in RAW 264.7 cells. (A) TNF-α production was measured in RAW 264.7 Cells were treated with different concentrations of MEOM (12.5, 25, 50 and 100 μg/ml), then incubated with LPS (10 μg/ml) for 24 h. (B) TNF-α production was measured after were primed with IFN-γ (0.5 ng/ml) for 2 h and then stimulated with LPS (5 μg/ml) in the presence of MEOM for 18 h. Data represents a means of three replicates with mean ± S.E.M and analysed using one-way ANOVA was followed by Dunnett's post hoc test.
***Data differed significantly ($P < 0.05$) when compared to the 10% DMSO-treated group.
*Data differed not significant as compared to the 10% DMSO-treated group.

TNF-α, pro-inflammatory cytokine produced from macrophages corresponding to inflammation. Therefore, the level of cytokine production, TNF-α was used as an indicator of macrophage response to LPS/IFN-γ was evaluated using ELISA technique.

Cells induced by LPS/IFN-γ, significantly increased the production of TNF-α in the DMSO-treated groups (control negative).

Treatment with MEOM had suppressed the production of the cytokines significantly, in a dose-dependent manner in the figure 8(A & B).

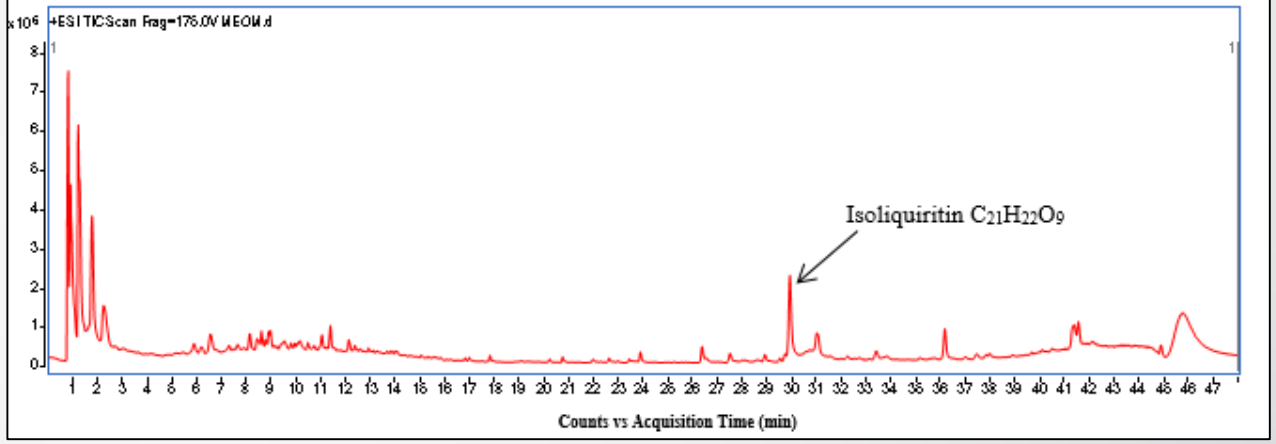
The inhibition of the pro-inflammatory cytokine activity of the highest concentration of extract (100 μg/mL) was noted to be 65.1% in figure 8A, and 82.0% in figure 8B, in comparison with ASA-treated groups (positive control); 89.6% (figure 8A) and 93.0% (figure 8B), respectively, as compared one-to-one to its respective LPS/IFN-γ-treated group.

RESULTS & DISCUSSION

UHPLC-Q-TOF/MS Acquisition Analysis

No	Proposed Compounds	Molecular Formula	Retention Time (min)	Molecular weight	Observed (m/z) $[M+H]^+$	Height (Peak)
1	Olivil 4″-o-glucopyranoside	$C_{26}H_{34}O_{12}$	0.791	150.03	151.04	303067
2	Protocatechuic acid	$C_7H_6O_4$	0.791	152.03	153.03	44749
3	Apigenin	$C_{15}H_{10}O_5$	0.798	267.94	268.95	26422
4	Carnosic acid	$C_{20}H_{28}O_4$	0.899	322.07	323.08	12323
5	4-O-caffeoylquinic acid	$C_{16}H_{18}O_9$	0.901	352.08	353.09	23526
6	4′-O-Methylepigallocatechin	$C_{16}H_{16}O_7$	0.966	320.07	321.08	29496
7	(-)-Epicatechin	$C_{15}H_{14}O_6$	0.967	290.06	291.07	11534
8	3′-Hydroxymelanettin	$C_{16}H_{12}O_6$	1.204	300.08	301.09	6268
9	Pyrogallol	$C_6H_6O_3$	1.615	126.04	127.04	42686
10	p-Coumaroyl tyrosine	$C_{18}H_{17}NO_5$	1.652	327.12	328.13	141863
11	Cinnamic acid	$C_9H_8O_2$	1.768	148.05	149.06	23685
12	Dihydrosinapic acid	$C_{11}H_{14}O_5$	1.943	226.09	227.10	13857
13	(-)-Epigallocatechin	$C_{15}H_{14}O_7$	7.274	306.07	307.08	15654
14	Matairesinol	$C_{20}H_{22}O_6$	8.495	372.10	373.11	38037
15	Dihydroferulic acid 4-O-glucuronide	$C_{16}H_{20}O_{10}$	8.968	372.10	373.11	21033
16	Esculetin	$C_9H_6O_4$	8.969	178.06	179.07	43220
17	Delphinidin 3-O-sambubioside	$C_{26}H_{29}O_{16}$	12.123	597.23	598.24	21681
18	1,3,5-Trimethoxybenzene	$C_9H_{12}O_3$	12.932	168.08	169.09	5667
19	Naringenin 7-O-glucoside	$C_{21}H_{22}O_{10}$	13.108	434.16	435.16	8366
20	Isoliquiritin	$C_{21}H_{22}O_9$	29.928	148.02	149.02	754267
21	Protocatechuic acid 4-O-glucoside	$C_{13}H_{16}O_9$	29.93	316.11	317.12	153808
22	Ceanothic acid	$C_{30}H_{46}O_5$	31.045	482.31	483.32	174633

FIGURE 3. LC-MS/MS chromatogram of MEOM by UHPLC-Q-TOF/MS



- ✓ UHPLC-Q-TOF/MS chromatogram showed 22 proposed compounds (Figure 3 & Table 3) in the MEOM.
- ✓ The compounds are classified as; 3 alkoloids, 5 polyphenols, 3 flavanones, 8 flavanoids, 2 nitrogen-containing compounds, 1 isoflavanoid & 1 flavanoid glycoside.
- ✓ isoliquiritin, a flavonoid glycoside compound; the strongest peak.

RESULTS & DISCUSSION

- ✓ The present study was exhibited the inhibition of pro-inflammatory mediators/cytokine via several inflammation pathways using *in vitro* models of inflammation by methanolic extract of *Opuntia monacantha* (MEOM).
- ✓ There was no basal NO production when cells were incubated with only the crude extract, 12.5-100 µg/mL without LPS/IFN-γ or >80% cells growth, which was considered noncytotoxic.
- ✓ The MEOM, significantly inhibit the LPS and/or IFN-γ induced -NO, -PGE_2, -COX-2, -iNOS, -TNF-α and -LOX production in dose-dependent manner at the different concentration (12.5, 25, 50 and 100µg/ml) in the RAW 264.7 macrophages cells.
- ✓ During the inflammatory process, iNOS and COX-2 are specifically expressed in the stimulation with LPS, IFN-γ and others pro-inflammatory cytokines (Yang *et al.*, 2012), that produce pro-inflammatory mediators such as NO and PGE_2 (Posadas I *et al.,* 2000). The MEOM possess anti-inflammatory effects by blocking the iNOS and COX-2 pathways-mediated inflammation, indicating the extract reduced the NO and PGE_2 production.
- ✓ TNF-α, is an inflammatory cytokine produced by macrophages/monocytes during acute inflammation (HT Indris *et al.*, 2000). The inhibition of TNF-α release by LPS and/or IFN-γ stimulated RAW 264.7 elicited by MEOM, suggesting that, *O. monacantha* may acts as antagonise of this cytokine or potential TNF-α inhibitors.

RESULTS & DISCUSSION

- ✓ Lipoxygenase (LOX) is mainly involved in the oxidation process of arachidonic acid (AA) into inflammatory mediators known as leukotrienes (LT's), which mediates the occurrence of inflammation (Noor Wahida Ismail *et al.*, 2017). The LOX inhibitory effect of MEOM significantly was found to be in a dose-dependent manner, respectively and suggest, as potential agent to attenuate the formation of gastric ulcer (Y Yonei and PH Guth *et al.*, 1991).

- ✓ Based on the UHPLC-Q-TOF/MS chromatogram, the strongest peak is identified as isoliquiritin, a flavonoid glycoside compound that has been reported to exhibit several pharmacological activities including antioxidant, anti-inflammatory, and anti-depression activities (Wang W *et al.*, 2007).

- ✓ This identified compound has a cytoprotective effect on corticosterone-induced neurotoxicity in PC12 cells, that related to its antioxidant action, inhibition of (Ca2+) overload, and inhibition of the mitochondrial apoptotic pathway and others (IB Slimen *et al.*, 2017).

CONCLUSION

The MEOM demonstrates the potential anti-inflammatory activity against LPS/IFN-γ-induced inflammation models, which could be attributed to the extract's;

i) anti-inflammatory activities,

ii) potential to regulate the PGE_2, COX-2, TNF-α, iNOs and LOX synthesis and,

iii) ability to work via pathways involving the NO. Moreover, this activity could be plausibly linked to the presence of inflammatory agents such as a flavonoid glycoside bio-compounds, which might act synergistically to produce the observed activity.