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ORIGINAL ARTICLE

Saponin of *Momordica cymbalaria* Exhibits Anti-Inflammatory Activity by Suppressing the Expression of Inflammatory Mediators in Lipopolysaccharide-Stimulated RAW264.7 Macrophages

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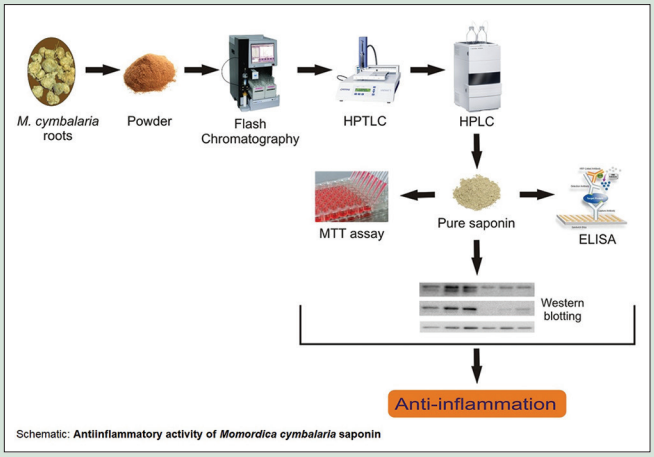
ABSTRACT

Background: Inflammation is an intricate biological process that commonly occurs in response to pathologic stimuli, and natural products have potential in healing inflammation. However, the anti-inflammatory of *Momordica cymbalaria* is not evaluated yet. **Objectives:** The anti-inflammatory mechanism of saponin of *M. cymbalaria* (SMC) was investigated in bacterial lipopolysaccharide (LPS)-stimulated RAW264.7 mouse macrophage cell line. **Methods:** The cytotoxicity of SMC on RAW264.7 cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay at 500, 250, 125, 62.5, 31.25, 15.625, 7.812, 3.906, and 1.953 μg/mL. For anti-inflammatory activity, RAW264.7 cells were stimulated with *Escherichia coli* LPS (1 μg/ml) in the presence or absence of SMC (50 μg/ml) for 16–24 h. Western blotting was carried to comprehend the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide (NO) synthase (iNOS) whereas expressions of the pro-inflammatory cytokines (interleukin [IL]-6, IL-1β, and tumor necrosis factor-alpha [TNF-α]) and prostaglandin E_2 (PGE_2) were studied by enzyme-linked immunosorbent assay (ELISA). NO production was estimated by Griess's method. Salicylic acid, a nonsteroidal anti-inflammatory drug, was used as a standard. **Results:** The saponin did not exert significant cytotoxicity on RAW264.7 cells. Western blot analysis revealed reduction in COX-2 and iNOS expression on SMC treatment. Production of PGE_2, IL-6, IL-1β, and TNF-α was also found to be reduced when analyzed by ELISA. NO levels were also lowered. **Conclusions:** The findings suggest that the SMC possesses potential anti-inflammatory activity by suppressing the expression of inflammatory mediators, COX-2, iNOS, PGE_2, and NO, and the cytokines in LPS-stimulated RAW264.7 cells.

Key words: Cyclooxygenase-2, interleukin-1 beta, inducible nitric oxide synthase, *Momordica cymbalaria,* prostaglandin E_2, tumor necrosis factor-alpha

SUMMARY

- Saponin of *Momordica cymbalaria* (SMC) was isolated and purified through flash chromatography and high-performance liquid chromatography. The *in vitro* cytotoxicity on RAW264.7 macrophage cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. *In vitro* anti-inflammatory activity was determined by studying the expressions of cyclooxygenase-2 (COX-2) and inducible nitric oxide (NO) synthase (iNOS) in RAW264.7 cells stimulated with *Escherichia coli* lipopolysaccharide (LPS) (1 μg/ml) by Western blotting. Pro-inflammatory cytokine (interleukin [IL]-6, IL-1β, and tumor-necrosis-factor-alpha [TNF-α]) production was studied by enzyme-linked immunosorbent assay. NO production was also studied. Results indicate that the saponin reduced the expressions of COX-2, iNOS, IL-6, IL-1β, and TNF-α in LPS-stimulated cells. NO production was also reduced. Thus, the SMC has been found to possess potential as an anti-inflammatory agent.



Schematic: **Antiinflammatory activity of *Momordica cymbalaria* saponin**

Abbreviations Used: IL-6: Interleukin-6; IL-1β: Interleukin-1 beta; TNF-α: Tumor necrosis factor-alpha; NO: Nitric oxide; COX-2: Cycloxygenase-2; LPS: Lipopolysaccharide; HPTLC: High-performance thin layer chromatography; TLC: Thin layer chromatography; HPLC: High-performance liquid chromatography; SMC: Saponin of *Momordica cymbalaria*; DMSO: Dimethylsulfoxide; EDTA: Ethylenediaminetetraacetate; SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TBST: Tris buffer saline tween; IgG-HRP: Immunoglobulin G-horse radish peroxidase; H_2O_2: Hydrogen peroxide; ELISA: Enzyme-linked immunosorbent assay.

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INTRODUCTION

Inflammation is a complex process mediated by the activation of various immune cells. Macrophages play a central role in mediating a number of different immunopathological phenomena during inflammation. Macrophages, when stimulated by bacterial endotoxin, produce inflammatory mediators including vasoactive amines, lipid mediators, pro-inflammatory cytokines (interleukins [ILs] and tumor-necrosis-factor alpha [TNF-α]), chemokines, proteolytic

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enzymes, prostaglandins, and reactive oxygen/nitrogen species, which have been implicated in the pathogenesis of tissue injury in spite of having valuable outcomes in inflammation.[1-3] Inducible nitric oxide (NO) synthase NOS (iNOS) sustainably produces a high proportion of NO, which is one of the most important inflammatory reactions in activated macrophages.[4] Furthermore, prostaglandin E_2 (PGE_2) produced by cycloxygenase-2 (COX-2), plays a fundamental role in the pathogenesis of acute and chronic inflammation.[5] A number of *in vivo* and *in vitro* studies have shown that iNOS inhibitors prevented the development of diseases such as experimental allergic encephalomyelitis, atherosclerosis, cancer, inflammatory bowel syndrome, and transplantation rejection.[6-8] Furthermore, inappropriate activation or upregulation of COX-2 is a trait of majority of inflammatory disorders,[9] and therefore, the inhibition of the abnormal upregulation of iNOS and COX-2 is considered a strategy for the treatment and prevention of inflammatory conditions and related diseases.

Momordica cymbalaria (*Cucurbitaceae*) is found in the south Indian states of Karnataka, Andhra Pradesh, and Tamil Nadu. The plant has also been named *Luffa tuberosa* (Roxb.) or *Momordica tuberosa* (Roxb.). It has been reported to possess Type I and II antidiabetic, antiovulatory, anti-implantation, abortifacient, and cardioprotective activities.[10-14] One of its variant species, *Momordica charantia* (bitter gourd), belonging to the same family, has attracted immense attention of the researchers in the past unraveling its antidiabetic potential. Unfortunately, *M. cymbalaria* has not been exploited much. We have reported previously that a saponin isolated from *M. cymbalaria* exhibits neuroprotective activity in diabetic peripheral neuropathy by inhibiting aldose reductase, a key enzyme of the polyol pathway,[15] and reduced the formation of IL-6, IL-1β, and TNF-α in mouse neuroblastoma cells.[16] The present study has been deliberated to investigate into the anti-inflammatory mechanism of the saponin of *M. cymbalaria* (SMC) in lipopolysaccharide (LPS)-stimulated RAW264.7 mouse macrophage cells.

MATERIALS AND METHODS

Plant material

The fresh roots of *M. cymbalaria* Fenzl were collected from Gadag district of Karnataka, India, and was identified and authenticated by the Department of Botany, Bangalore University, Bangalore, India (voucher No. 18122003).

Extraction and isolation of saponin

Each 100 g powder was subjected to extraction with 1000 mL methanol in a reflux condenser for three cycles of 7 h and each till the volume reduced to half. Extract was filtered through Whatman filter paper No. 1 and evaporated to dryness. Further isolation and purification were achieved through flash chromatography (CombiFlash® Rf, Teledyne Technologies Inc., ISCO, USA) and eluted with methanol: chloroform (80:20) with a flow rate of 20 mL/min using RediSep® Silica preparative column. Eluted saponin fraction was first subjected to Liebermann-Burchard test for triterpenoids and then evaluated by high-performance thin layer chromatography (HPTLC, Camag, Switzerland) on 10 cm × 10 cm silica gel 60-GF_{254} plates (Merck). Sample and ursolic acid standard (U6753-100MG, Sigma) were applied 10 µL each to the layers as 10-mm wide bands, 15 mm from the bottom and sides of the plates (CAMAG Linomat 5 applicator). The plate was developed to a distance of 80 mm with the same mobile phase as TLC in a Camag twin trough glass chamber previously saturated with mobile phase (chloroform: methanol: 95:5) vapor. The plate was air-dried, derivatized by spraying with anisaldehyde-sulfuric acid, and heated at 105°C for 5 min. The bands were visualized by scanning the plate in an ultraviolet (UV) scanner (Camag TLC Scanner III) at 560 nm. The fraction was further chromatographed in reverse-phase high-performance liquid chromatography (HPLC, Agilent 1120 LC) using a C_{18} column (5 µm), UV detector and eluted with methanol: chloroform (80:20) with a flow rate of 1 mL/min against ursolic acid standard (U6753-100MG, Sigma).

Cell culture and cytotoxicity assessment

RAW264.7 mouse monocyte-macrophage cell line was obtained from the National Centre of Cell Science, Pune, India, and grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (HiMedia, Mumbai, India), 50 IU/ml penicillin, 25 µg/ml streptomycin, and 2.5 µg/ml amphotericin B. The assessment of cytotoxicity of SMC was done by the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan. Cells were seeded in a 96-well plate (5–10 × 10^3 cells/well) and left to attach on to the substrate overnight before being exposed to SMC and incubated at 37°C for 24 h. The cells were then treated with various concentrations of SMC (500, 250, 125, 62.5, 31.25, 15.625, 7.813, 3.906, and 1.953 µg/ml) and incubated for 36 h. After 36 h, 50 µL MTT (5 mg/mL) solution was added to each well and the cells were incubated in the dark at 37°C for an additional 4 h. Thereafter, the medium was removed, the formazan crystals were dissolved in 200 µl dimethylsulfoxide and 25 µl glycine buffer (pH 10.5), and the absorbance was measured at 570 nm in a microplate reader. The percentage of cell viability was calculated.

In vitro anti-inflammatory activity

RAW264.7 cells were stimulated with bacterial (*Escherichia coli*) LPS (L5418-2ML, Sigma-Aldrich, India) at a final concentration of 1 µg/ml per well and incubated for 16–24 h. The phytoconstituent (SMC) was added 50 µg per well. One "growth control," "LPS control," and "positive control" (salicylic acid, 50 µM) were maintained for comparison of data. The cells were incubated for 18 h. After incubation, cells adhering were lysed using ice-cold cell lysis buffer containing 1X Triton-X-100 (1%), Tris-HCl (50 mM, pH 7.4), ethylenediaminetetraacetate (5 mM), NaCl (150 mM), and SigmaFast™ protease inhibitor cocktail (S8830, Sigma-Aldrich). The lysates were centrifuged at 10,000 ×*g* for 5 min at 4°C to sediment the cell debris. The samples were concentrated to 0.5–0.8 ml by ultrafiltration using centrifugal filter devices (Millipore's Amicon Ultracel-3K; 30 kDa cutoff, regenerated cellulose). Total protein concentration was determined by bicinchoninic acid assay kit (BCA1, Sigma-Aldrich). The protein concentrate was stored at −80°C for the estimation of PGE_2, COX-2, and iNOS. For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), 30 µg of total protein per sample was loaded. The culture supernatant (medium) was also collected, before cell lysis, and stored at −80°C for measurement of NO and IL-6, IL-1β, and TNF-α production.

Western blot analysis of cyclooxygenase-2 and inducible nitric oxide synthase

The protein separation was done by SDS-PAGE (7.5% Mini-Protean® TGX™ gel, Bio-Rad) and blotted on to nitrocellulose membranes (0.22 µm, Bio-Rad, USA). The membranes were blocked with 5% nonfat-dried milk in tris buffer saline tween for 90 min and incubated overnight at 4°C on a gel rocker with anti-COX-2 monoclonal antibodies (1:750; Abcam ab62331, USA), anti-iNOS monoclonal antibodies (1:1000; abcamab178945, USA), and anti-β-actin monoclonal antibodies (1:1000; Abcam ab190476, USA) followed by anti-rabbit immunoglobulin G-horse radish peroxidase (IgG-HRP) conjugate (1:5000; Abcam ab6721, USA). Substrate buffer was added containing 1 mg/mL 3',3',5',5'-tetramethylbenzidine and 0.01% hydrogen peroxide and stop solution (2.5 N H_2SO_4). The bands developed were

scanned densitometrically and expression of COX-2 and iNOS was normalized on the basis of β-actin levels (loading control).

Prostaglandin E_2 immunoassay

PE_2 was assayed by antigen capture enzyme-linked immunosorbent assay (ELISA) using PGE_2 ELISA kit (ab133021; Abcam, USA).

Pro-inflammatory cytokine (interleukin-6, interleukin -1 beta, and tumor necrosis factor-alpha) immunoassay

IL-6, IL-1β, and TNF-α were assayed by antigen capture ELISA. Primary antibodies of IL-6 (sc-1266; SCBT), IL-1β (sc-7884), and TNF-α (sc-1348) were used for antigen capture. Cell culture supernatant and standard cytokines IL-6 (hBA-184; sc-4597), IL-1β (hBA-153; sc-4592), and TNF-α (hBA-158; sc-4564) were added followed by addition of detecting secondary monoclonal antibodies (anti-IL-6, anti-IL-1β, and anti-TNF-α; 1:1000, SCBT, USA). HRP-conjugated secondary antibody (1:5000, rabbit anti-mouse IgG-HRP; sc-358914) was added followed by the substrate.

Assay for nitric oxide production

The growth medium was equally blended with Griess's reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride) followed by incubation at room temperature for 10 min. The $A_{540\ nm}$ was recorded by an ELISA reader (Readwell Touch, Robotnik, India). Nitrite estimation was done against a sodium nitrite standard.[17]

Statistical analysis

The results are expressed as mean ± standard error of mean (SEM) and one-way analysis of variance (ANOVA) followed by Dunnett's test was used to determine statistical significance. $P < 0.001$ was considered statistically significant.

RESULTS

Saponin isolation and characterization

The saponin fraction obtained from flash chromatography tested positive for Liebermann-Burchard test (pink color obtained) indicating the presence of pentacyclic triterpenoid and was resolved on HPTLC against ursolic acid (a pentacyclic triterpenoid) standard [Figure 1]. The saponin had almost identical retention factor ($R_f = 0.69$) as that of ursolic acid standard ($R_f = 0.68$). A single spot with identical R_f as that of the standard indicates isolation of the saponin in pure form (Lane 1 = ursolic acid [L1]; Lane 2 = saponin fraction [L2]).

The reverse-phase HPLC chromatograms of ursolic acid and saponin [Figure 2a and b] show near identical retention times (ursolic acid $R_t = 4.723$ min and the isolated saponin $R_t = 4.727$ min). The retention pattern of the saponin matches with that of the standard which indicates its similarity with the standard.

Assessment of cytotoxicity of saponin of *Momordica cymbalaria* on RAW264.7 cells

As shown in Table 1, it was found that the saponin did not exert significant cytotoxicity on the cells. Even at the highest test concentration of 500 μg/ml, 81.36 ±0.2871% viability of cells was evident.

In vitro anti-inflammatory activity

The expression of inflammatory mediators COX-2, PGE_2, and iNOS was found to be increased in the cells challenged with LPS in comparison to the normal control ($P < 0.001$). However, upon treatment with SMC (50 μg) and salicylic acid (50 μM), the production of the mediators was found to be reduced in comparison to the LPS control ($^{\#\#}P < 0.01$). The response of SMC was found to be comparable to the positive control salicylic acid [Figure 3a-d].

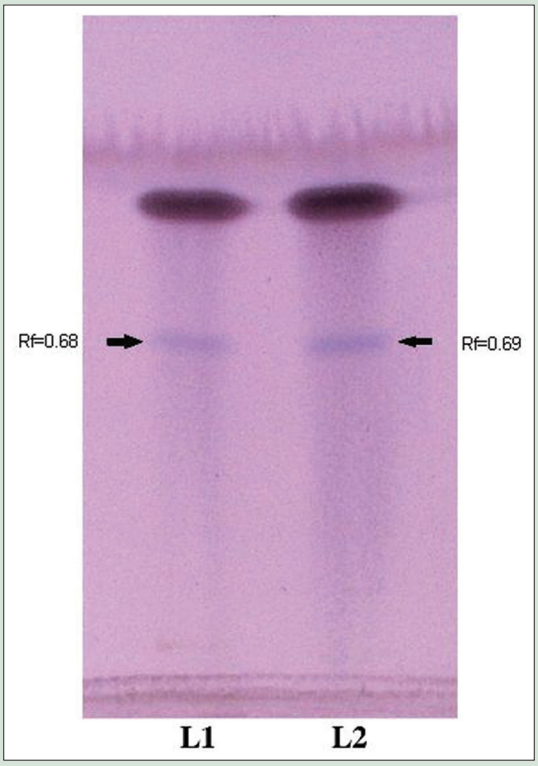

Figure 1: High-performance thin layer chromatography of saponin fraction collected through flash chromatography (Lane 2) against ursolic acid standard (Lane 1) followed by densitometric scanning at 560 nm

Pro-inflammatory cytokine (interleukin-6, interleukin -1 beta, and tumor necrosis factor-alpha) immunoassay

Assessment of the anti-inflammatory activity of SMC was done on RAW264.7 cells challenged with LPS. It was found that the production of all three pro-inflammatory cytokines, viz., IL-6, IL-1β, and TNF-α, was increased considerably upon LPS treatment. However, treatment with SMC has significantly suppressed the production of the cytokines in comparison to the LPS control ($P < 0.001$). The decrement in cytokine production by SMC was found to be prominent than that of salicylic acid ($P < 0.05$) [Figure 4].

Effect on nitric oxide production

NO production was augmented in cells treated with LPS (1 μg/ml) in comparison to the normal control ($P < 0.001$). Upon treatment with SMC and salicylic acid, the NO production was reduced significantly ($P < 0.001$) in comparison to LPS control [Figure 5].

DISCUSSION

Medicinal plants have always gained ethnopharmacological importance in the treatment of inflammation and pain. A few to mention here are *Zingiber officinale* (ginger),[18,19] *Aloe barbadensis* (Aloe vera),[20] *Withania somnifera* (Ashwagandha),[21] *Allium sativum* (garlic),[22] *Curcuma*

longa (turmeric), etc.[23,24] We have shown in our earlier work that *M. cymbalaria* saponin has reduced the formation of pro-inflammatory cytokines, viz., IL-6, IL-1β, and TNF-α, in high glucose condition, thereby ameliorating the ill effects of diabetic neuropathy.[16] However, its direct effect on LPS-stimulated macrophages, altering the expression levels of cytokines and other inflammatory mediators (COX-2, iNOS, and PGE_2), was not investigated, and hence, the rationale of this study. The saponin isolated is believed to have structural analogy to ursolic acid, a pentacyclic oleanane-type triterpenoid, the reason being that the R_t (HPLC) and R_f (HPTLC) of both are near-identical. It was also found to have no significant cytotoxicity on RAW264.7 macrophages as >80% of the cell population remained viable at the highest test concentration, suggesting its safety. In the present study, we showed that SMC effectively inhibits LPS-stimulated production of COX-2, iNOS, PGE_2, IL-6, IL-1β, TNF-α, and NO by the RAW264.7 macrophages. It is a well-established fact that NO and PGE_2 are the main macrophage-derived inflammatory mediators[25,26] that can be induced by the expressions of iNOS and COX-2, respectively, as a part of the innate immune system, and the inhibition of NO and PGE_2 overexpression might be used as a therapeutic tool

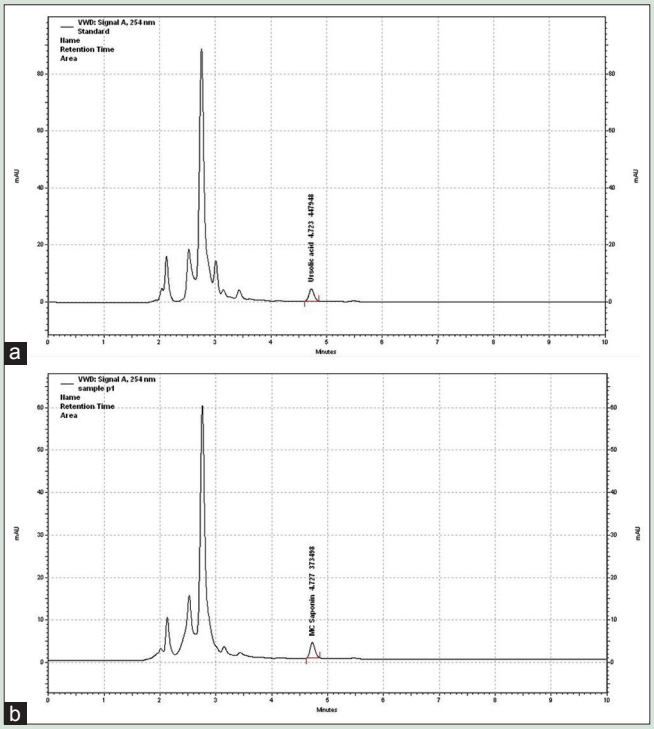

Figure 2: Reverse-phase high-performance thin layer chromatograms of ursolic acid standard, retention time of 4.723 min (a) and isolated saponin from *Momordica cymbalaria*, retention time of 4.727 min (b)

Table 1: Cytotoxicity of saponin of *Momordica cymbalaria* at different concentrations on RAW264.7 cells

Concentration (µg/mL)	Viability (%)
500	81.36±0.287
250	84.82±0.430
125	86.80±0.323
62.5	88.29±0.243
31.25	90.97±0.213
15.625	92.32±0.291
7.813	93.45±0.232
3.906	97.20±0.255
1.953	98.48±0.216

Values are expressed as mean±SEM (n=6). Production size: At column width. SEM: Standard error of mean

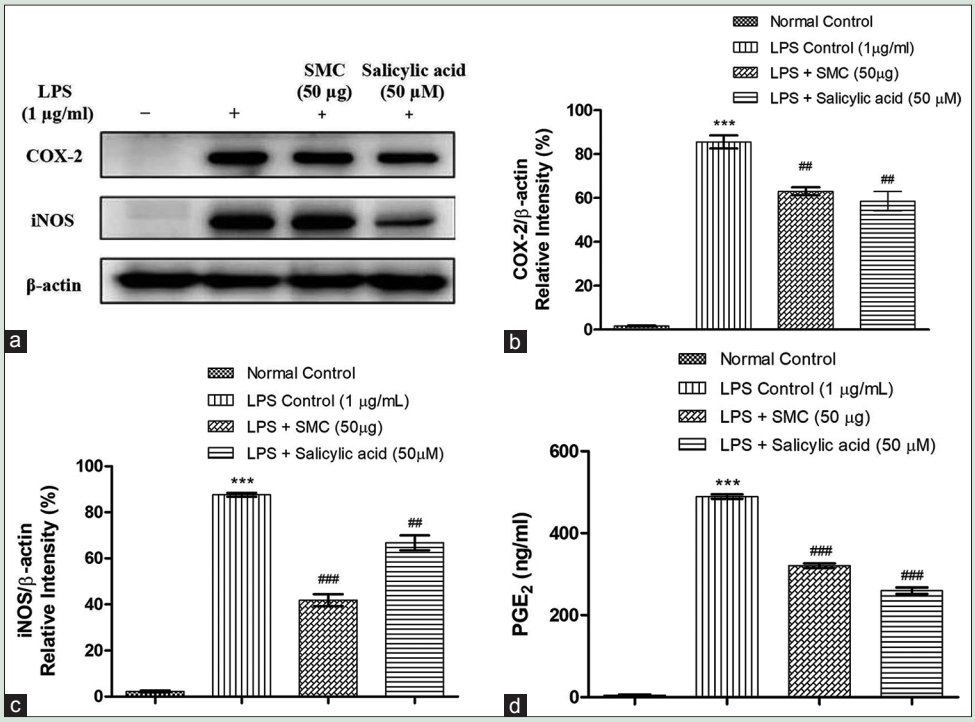

Figure 3: Effect of saponin of *Momordica cymbalaria* on the inhibition of LPS-induced expression of COX-2, iNOS, and PGE_2 in RAW264.7 cells. (a) Western blot; (b and c) densitometric analysis of COX-2 and iNOS; and (d) ELISA of PGE_2. Values are expressed as mean ± standard error of the mean (n = 4). ***P < 0.001 compared with the normal control; ###P < 0.001, ##P < 0.01 compared with the LPS control. COX-2: Cycloxygenase-2; LPS: Lipopolysaccharide; iNOS: Inducible nitric oxide synthase; PGE_2: Prostaglandin E_2; ELISA: enzyme-linked immunosorbent assay

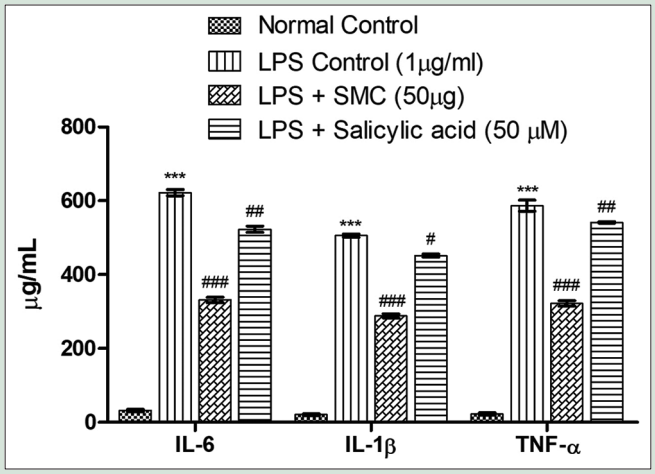

Figure 4: Effect of saponin of *Momordica cymbalaria* on the production of inflammatory cytokines IL-6, IL-1β, and TNF-α in RAW264.7 cells challenged with LPS. Values are expressed as mean ± standard error of the mean ($n = 6$). ***$P < 0.001$ compared with the normal control; ###$P < 0.001$, ##$P < 0.01$, #$P < 0.05$ compared with the LPS control. IL-6: Interleukin-6; IL-1β: Interleukin-1 beta; TNF-α: Tumor necrosis factor-alpha; LPS: Lipopolysaccharide

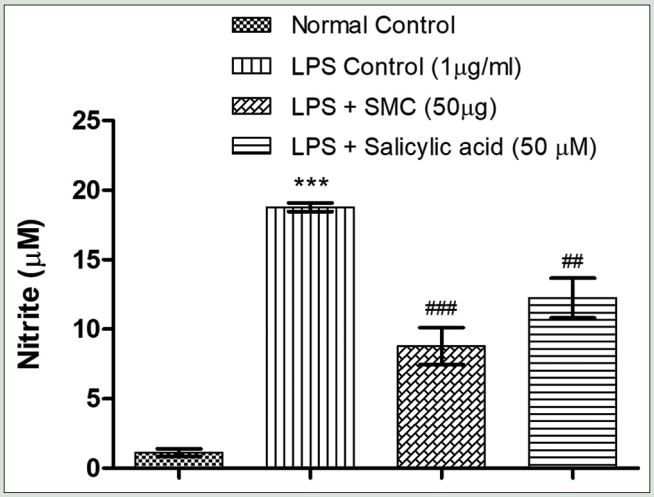

Figure 5: Effect of saponin of *Momordica cymbalaria* on LPS-induced NO production. Values are expressed as mean ± standard error of the mean ($n = 6$). ***$P < 0.001$ compared with the normal control; ###$P < 0.001$, ##$P < 0.01$ compared with the LPS control. NO: Nitric oxide; LPS: Lipopolysaccharide

to treat inflammatory diseases. The Western blot findings of this study reveal that the saponin reduced the expression of COX-2 and iNOS as well as PGE_2 and NO in LPS-primed macrophages. Besides, it was also reported that TNF-α is an inflammatory cytokine, which is considered as an endogenous mediator in LPS-induced shock.[27] Abnormalities in the production or function of cytokines, such as TNF-α and IL-1β, may play roles in many inflammatory reactions.[28] TNF-α is mainly synthesized by the monocytes, macrophages, and T-cells and has roles to play in the innate immune response. As such, it is a potent activator of macrophages and can stimulate the production or expression of IL-6, IL-1β, PGE_2, collagenase, and adhesion molecules. Thus, the inhibition of cytokine production or function is a key mechanism in the control of inflammation. In the present study, we found that SMC significantly inhibited the production of the pro-inflammatory cytokines IL-6, IL-1β, and TNF-α in macrophages stimulated by LPS. NF-κB is a pleiotropic regulator of various genes involved in immune and inflammatory responses. It has been shown that NF-κB activation is a key transcription factor which activates several cellular signal transduction pathways that are implicated in the production of iNOS, COX-2, and various cytokines.[5,29] The promoter region of the murine gene encoding iNOS and COX-2 contains NF-κB-binding motifs. It has been reported that binding of NF-κB to its binding sites upstream of the iNOS and COX-2 promoters plays an important role in the LPS-induced upregulation of the iNOS and COX-2 genes.[30] Ginsenoside Rd, a triterpenoid saponin of *Panax ginseng*, was found to inhibit expression of iNOS and COX-2 by suppressing NF-κB in LPS-stimulated RAW264.7 cells and mouse liver.[31] As the SMC is a triterpenoid and shares structural similarity with *Panax ginseng* saponin as well, we hypothesize that SMC might have blocked the LPS-mediated activation of NF-κB, leading to inhibition of other inflammatory mediators.

CONCLUSIONS

The findings demonstrate that the SMC attenuated LPS-stimulated inflammatory responses in RAW264.7 macrophages by inhibiting the overexpression of various mediators of inflammation, viz., COX-2, iNOS, PGE_2, IL-6, IL-1β, TNF-α, and NO. These findings provide evidence that SMC possesses potentially useful anti-inflammatory activities.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Laskin DL, Pendino KJ. Macrophages and inflammatory mediators in tissue injury. Annu Rev Pharmacol Toxicol 1995;35:655-77.
2. MacMicking J, Xie QW, Nathan C. Nitric oxide and macrophage function. Annu Rev Immunol 1997;15:323-50.
3. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol 2011;31:986-1000.
4. Zhao F, Chen L, Bi C, Zhang M, Jiao W, Yao X, *et al. In vitro* anti-inflammatory effect of picrasmalignan A by the inhibition of iNOS and COX-2 expression in LPS-activated macrophage RAW 264.7 cells. Mol Med Rep 2013;8:1575-9.
5. Baldwin AS Jr. The NF-kappa B and I kappa B proteins: New discoveries and insights. Annu Rev Immunol 1996;14:649-83.
6. Mann JR, Backlund MG, DuBois RN. Mechanisms of disease: Inflammatory mediators and cancer prevention. Nat Clin Pract Oncol 2005;2:202-10.
7. Song SB, Tung NH, Quang TH, Ngan NT, Kim KE, Kim YH, *et al.* Inhibition of TNF-α-mediated NF-κB transcriptional activity in hepG2 cells by dammarane-type saponins from *Panax ginseng* leaves. J Ginseng Res 2012;36:146-52.
8. Karin M, Cao Y, Greten FR, Li ZW. NF-kappaB in cancer: From innocent bystander to major culprit. Nat Rev Cancer 2002;2:301-10.
9. Hilmy M, Campbell R, Bartlett JM, McNicol AM, Underwood MA, McMillan DC. The relationship between the systemic inflammatory response, tumour proliferative activity, T-lymphocytic infiltration and COX-2 expression and survival in patients with transitional cell carcinoma of the urinary bladder. Br J Cancer 2006;95:1234-8.
10. Koneri RB, Samaddar S, Ramaiah CT. Antidiabetic activity of a triterpenoid

saponin isolated from *Momordica cymbalaria* Fenzl. Indian J Exp Biol 2014;52:46-52.
11. Firdous M, Koneri R, Sarvaraidu CH, Harish M, Shubhapriya KH. NIDDM antidiabetic activity of saponins of *Momordica cymbalaria* in streptozotocin-nicotinamide NIDDM mice. J Clin Diagn Res 2009;3:1460-5.
12. Koneri R, Balaraman R, Saraswati CD. Antiovulatory and abortifacient potential of the ethanolic extract of roots of *Momordica cymbalaria* Fenzl in rats. Indian J Pharmacol 2006;38:111-4.
13. Koneri R, Saraswati CD, Balaraman R, Ajeesha EA. Anti-implantation activity of the ethanolic root extract of *Momordica cymbalaria* Fenzl in rats. Indian J Pharmacol 2007;9:90-6.
14. Koneri R, Balaraman R, Hariprasad, Vinoth KM, Ali A. Cardioprotective effect of *Momordica cymbalaria* Fenzl in rats with isoproterenol-induced myocardial injury. J Clin Diagnos Res 2008;2:699-705.
15. Suman S, Raju KB, Abhinaya B, Chandrasekhar KB. Oleanane-type triterpenoid saponin of *Momordica cymbalaria* exhibits neuroprotective activity in diabetic peripheral neuropathy by affecting the polyol pathway. Int J Pharm Sci Res 2016;7:1000-8.
16. Suman S, Raju KB, Sunil KS, Chandrasekhar KB. Protective effect of saponin of *Momordica cymbalaria* Fenzl on high-glucose induced neuropathy in NB-41A3 mouse neuroblastoma cells. Int J Pharm Pharm Sci 2016;8:229-35.
17. Kang CH, Choi YH, Choi IW, Lee JD, Kim GY. Inhibition of lipopolysaccharide-induced iNOS, COX-2, and TNF-α expression by aqueous extract of *Orixa japonica* in RAW 264.7 cells via suppression of NF-κB activity. Trop J Pharma Res 2011;10:161-8.
18. Mascolo N, Jain R, Jain SC, Capasso F. Ethnopharmacologic investigation of ginger (*Zingiber officinale*). J Ethnopharmacol 1989;27:129-40.
19. Mustafa T, Srivastava KC. Ginger (*Zingiber officinale*) in migraine headache. J Ethnopharmacol 1990;29:267-73.
20. Altaee N, Kadhim MJ, Hameed IH. Detection of volatile compounds produced by *Pseudomonas aeruginosa* isolated from UTI patients by gas chromatography-mass spectrometry. Int J Curr Pharm Rev Res 2017;7:462-70.
21. Kadhim MJ. *In vitro* antifungal potential of *Acinetobacter baumannii* and determination of its chemical composition by gas chromatography-mass spectrometry. Der Pharma Chem 2016;8:657-65.
22. Ban JO, Oh JH, Kim TM, Kim DJ, Jeong HS, Han SB, *et al.* Anti-inflammatory and arthritic effects of thiacremonone, a novel sulfur compound isolated from garlic via inhibition of NF-kappaB. Arthritis Res Ther 2009;11:R145.
23. Lao CD, Ruffin MT 4th, Normolle D, Heath DD, Murray SI, Bailey JM, *et al.* Dose escalation of a curcuminoid formulation. BMC Complement Altern Med 2006;6:10.
24. Jurenka JS. Anti-inflammatory properties of curcumin, a major constituent of curcuma longa: A review of preclinical and clinical research. Altern Med Rev 2009;14:141-53.
25. Vodovotz Y, Kim PK, Bagci EZ, Ermentrout GB, Chow CC, Bahar I, *et al.* Inflammatory modulation of hepatocyte apoptosis by nitric oxide: *In vivo*, *in vitro*, and *in silico* studies. Curr Mol Med 2004;4:753-62.
26. Moncada S, Higgs EA. Nitric oxide and the vascular endothelium. Handb Exp Pharmacol 2006;176:213-54.
27. Dinarello CA. Cytokines as endogenous pyrogens. J Infect Dis 1999;179 Suppl 2:S294-304.
28. De Nardin E. The role of inflammatory and immunological mediators in periodontitis and cardiovascular disease. Ann Periodontol 2001;6:30-40.
29. Baeuerle PA, Henkel T. Function and activation of NF-kappa B in the immune system. Annu Rev Immunol 1994;12:141-79.
30. Lee AK, Sung SH, Kim YC, Kim SG. Inhibition of lipopolysaccharide-inducible nitric oxide synthase, TNF-alpha and COX-2 expression by sauchinone effects on I-kappaBalpha phosphorylation, C/EBP and AP-1 activation. Br J Pharmacol 2003;139:11-20.
31. Kim DH, Chung JH, Yoon JS, Ha YM, Bae S, Lee EK, *et al.* Ginsenoside rd inhibits the expressions of iNOS and COX-2 by suppressing NF-κB in LPS-stimulated RAW264.7 cells and mouse liver. J Ginseng Res 2013;37:54-63.