Original Research Article

Modulatory effects of leave and fruit extracts of *Ficus sycomorus* on cytostatic and inflammatory mediators in monocultures and cocultures of human keratinocyte (HaCat) and human monocyte (THP-1) cell lines

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ABSTRACT

Background: Ficus sycomorus (FS) is one of the recommended antipsoriatic medicinal plants in the traditional Greco-Arab herbal medicine. Yet there is very limited knowledge available about its action mechanisms.
Aims: Here, cytotoxic, cytostatic, and anti-inflammatory effects of water/ethanolic extracts of FS leaves and fruits were evaluated to test their role in the traditionally known antipsoriatic properties.
Place and Duration of Study: All the experiments were done in the Department of Biology and Biotechnology, Arab American University-Palestine in 2020. Plants were collected from the Northern region of the West Bank/Palestine during the fall months in 2019 and given (Voucher code: Pharm-PCT-1030) at An-Najah National University.
Methodology: MTT assay was used to evaluate cytostatic and cytotoxic effects of FS extracts in human skin keratinocyte cell line (HaCaT), human monocytic cell line (THP-1)-derived macrophages, and their co-cultures. Commercial ELISA kits were applied to measure the cytokine levels.
Results: Both extracts exhibited cytostatic effects with IC 50 of 656 µg/mL and 886 µg/mL for HaCat and coculture, respectively. Leaves and fruits extracts significantly reduced dose-dependently the LPS-induced NO production by THP-1-derived macrophages from 65 µM to 19 µM and 16 µM, respectively. The fruit extracts showed higher effects than the leaf extracts and reduced the TNF-α levels from 709 pg/mL to 208 pg/mL. The fruit extracts increased the production levels of IL-10 from 74 pg/mL to 90 pg/mL.
Conclusion: FS extracts probably exert their antipsoriatic effects through cytostatic effects and modulation of the production levels of pro-inflammatory and anti-inflammatory cytokines.

Keywords: Psoriasis, skin diseases, inflammation, Greco-Arab herbal medicine.

1. INTRODUCTION

With a worldwide incidence of 2-4%, psoriasis is one of the most common chronic inflammatory, stress-related sicknesses that affect the physical and mental well-being of patients [1, 2]. It is characterized by an abnormal cell cycle of epidermal cells that leads to epidermal hyperproliferation and altered maturation of these cells, vascular alterations, and inflammation [1, 2] . The pathogenesis of psoriasis is highly complex, that includes keratinocytes, inflammatory cells, pro-inflammatory cytokines, and growth factors [3, 4]. Recently published data have delivered new understandings of the cellular and molecular

pathways involved in the pathogenesis of psoriasis. Data obtained in several studies showed that patients with psoriasis had significantly higher blood levels of TNF-α, interferon (IFN)- γ, IL-2, IL-6, IL-7, IL-8, IL-12, IL-17, IL-18, and vascular endothelial growth factor (VEGF) than did healthy controls. Increased IL-12, IL-17, IL-18, TNF-α, IFN, and VEGF serum levels were associated with psoriasis area and severity index (PASI). Following psoriasis therapy, these cytokine levels in the serum reduced. In contrast, psoriasis patients had lower blood levels of the anti-inflammatory cytokine IL-10, which had a negative correlation with PASI [5-7] .IL-10 has a significant impact on immunomodulation [8-10]. It stimulates the development of type 2 cytokines via reducing the IFN- production by T cells and natural killer cells predominantly through the suppression of IL-12 synthesis in accessory cells. Moreover, it decreases pro-inflammatory cytokine production and antigen-presenting capability of macrophages and dendritic cells. Interestingly, intravenous administration of IL-10 inhibited the production of pro-inflammatory cytokine in patients with Crohn's and other inflammatory bowel diseases (IBDs)[9-12]. Therefore, beneficial effects of IL-10 therapy in psoriasis also may be expected.

A variety of treatments are currently used to manage the severity of symptoms and to improve a patient's quality of life. Corticosteroids and broadband ultraviolet B (UVB) are the most commonly prescribed topical drugs for psoriasis. However, these treatments are all associated with significant adverse side effects [1, 2, 13].

Medicinal plants represent a promising source of effective therapy for treating psoriasis. Antipsoriatic medicinal plants and phytomedicines are believed to provide some benefits, such as being natural, having fewer side effects, and avoiding unhappiness with conventional therapy. The structural variety and several modes of action of the herbal products have made it possible for the synergistic activity to reduce psoriasis [14]. Therefore, many plants that have been traditionally used by indigenous healers worldwide for the treatment of skin diseases are currently under investigation to assess how effective they could be at treating psoriasis [15-19].

The Mediterranean region is such an example of an ongoing process of cultural collision. The development of modern medicine in Europe was based historically on the early Greek knowledge preserved and developed by Arab culture. Greco-Arab medicine is highly rich and diverse in knowledge. Psoriasis is managed with great efficacy and safety employing traditional Greco-Arab and Islamic medicine [16-18]. Psoriasis patients in Palestine were reported to use 33 medicinal plants belonging to 26 families [20]. The crude extracts from plant or animal sources contain different classes of bioactive compounds. These bioactive substances may work synergistically to cure illnesses as a result of their combination found in the extracts. However, many plants used in this Greco-Arab medicine system have not been investigated as a source for new medicines. Yet there is very limited knowledge is available about the action mechanisms of these plants [15-19].

FS is a member of the Moraceae family which includes 40 genera and more than 800 species. FS is primarily found to contain terpenoids, flavonoids, and phenolic compounds. Traditional Greco-Arab and Islamic medicine both employ FS to manage digestive, neurological, cardiovascular, and sexual diseases, skin diseases, infections, diabetes, and cancer [21]. The white latex is used to treating burns, ulcers, warts, and inflammation. The latex is often used to prevent infection and to enhance healing among Africans. Water boiled bark treats scrofula, sore throats, respiratory illnesses, and chest pain. Leaves are used as an antidote for snake bites, roots are known for their laxative effects, fruits help breastfeeding, and seeds prevent bacterial infections [21, 22].

To understand the role of pro-inflammatory as well as anti-inflammatory cytokine in traditionally known antipsoriatic effects of FS, we aimed in this *in vitro* study to evaluate the cytotoxicity, cytostatic in HaCat and HaCat/THP-1 cells and the anti-inflammatory mechanism of FS leaves and fruits by measuring the release of IL-10, IL-6, TNF-α, and nitric oxide (NO) from the human THP-1-derived macrophages.

2. MATERIAL AND METHODS

Plants material

Leaves and fruits of FS plant were collected from the Northern region of the West Bank/Palestine during the fall months (Table 1). The botanical identity of the collected FS (voucher code: Pharm-PCT-1030) materials was confirmed by Nidal Jaradat (An-Najah National University). The samples were delivered to the Pharmacognosy lab and identified with the appropriate voucher specimen codes. Samples were crushed into a fine powder and kept at 4 °C until extraction after being air-dried for 10 days.

Table 1. Ethnobotanical data, collection time, parts used of the plants and solvent used in the study.

Plant species	Common name/Arabic name	Used parts	Time of collection	Solvent
Ficus sycomorus **(Voucher code: Pharm-PCT-1030)**	Sycamore fig/ Jummayz	Leaves	September, 2019 2nd week	50% ethanol/water
		Fruits	October 2019 3rd week	50% ethanol/water

Plant extracts

Sixteen grams of each powder were soaked in 100 mL of 50% ethanol/water solution with continuous mixing for 20 min at 70 °C. Subsequent centrifugation at 4000 rpm for 15 min was performed three times with the extracts at room temperature. The supernatants were condensed using a rotating vacuum evaporator after being passed through a 0.2 m filter. The yield of the extracts was determined to be 12.2% for the leaves and 12.8% for the fruits. The powders were kept at -70°C for future analysis.

Cell culture

Roswell Park Memorial Institute media (RPMI-1640) was used to grow the human monocytic cell line THP-1, which was obtained from a patient with acute monocytic leukemia (ATCC: TIB-202), and Dulbecco's Modified Eagle Medium (DMEM-5671) was used to grow the human keratinocytes cell line HaCaT. Both media types contained a high glucose concentration (4.5 g/L) and were supplemented with 10% (vol/vol) fetal calf serum (FCS), 1% nonessential amino acids, 1% L-glutamine, 100 U/mL penicillin, and 10 µg/mL

streptomycin (Sigma-Aldrich). Cells from both cell lines were cultured at 37 °C, pH 7.4, in a humidified environment containing 5% CO2.

Cell differentiation

With the aid of 0.1 µM/mL medium Vitamin D3 and 100 ng/mL medium phorbol-12-myristate-13-acetate (PMA) (Sigma-Aldrich, USA), THP-1 cells were differentiated into macrophages in 96-micro-titer plates for 24 h.

Cytotoxic and cytostatic effects in monoculture and co-culture system systems

Cytotoxic effects: 20,000 cells/100 µl were seeded in 96-microtiter plates for the monoculture system. For the co-culture system, 10,000 THP-1 cells were differentiated for 24 h (as mentioned in 2.4). Then the resulted differentiated THP-1-derived macrophages were washed three times with PBS and 10,000 HaCaT cells were added. Twenty-four h later, cells were treated with varying concentrations of FS (0-1000 µg/mL). The MTT assay was then performed.
Cytostatic effects: 5,000 cells/100 µl media from each cell line were seeded in 96-microtiter plates for cytostatic tests for the monoculture system. For the co-culture system, 2500 THP-1 cells were differentiated (as mentioned in 2.4) for 24 h then washed three times with PBS and replaced with 100 µL DMEM medium and 2500 HaCaT cells were added. Twenty-four h later, cells were treated for 72 h with varying concentrations of FS (0-1000 µg/mL). The MTT assay was then performed.

MTT Assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test is based on the conversion of the yellow tetrazolium salt into a dark purple insoluble crystalline formazan by NAD(P)H-dependent oxidoreductase enzymes in live cells. This test was applied to assess cell viability. The MTT assay was performed according to Kaadan et al., [23]. Briefly, cells were seeded in 96-microtiter plates as described above for cytostatic and cytotoxic measurements. At 100 µL MTT solution (0.5 mg/mL MTT in serum-free medium) was added at the completion of the treatment period and was incubated for 4 h at 37 °C and 5% CO2 in a chamber. The medium was withdrawn, cells were washed three times with PBS, and the formazan crystals were dissolved in 100 µL of acidic isopropanol (0.08 N HCL) for 20 min. At 570 nm, an ELISA reader (680, BioRad, USA) was used to quantify the MTT formazan's absorbance. The percentage difference in absorbance between treated and untreated cells was used to define cell viability.

Determination of NO production

As previously mentioned, test samples' capacity to inhibit NO generation in LPS-activated macrophages was evaluated [24]. The concentrations of secreted NO into the cell culture media were measured with the Griess reagent. With the aid of this reagent, nitrite is turned into a purple azo molecule that can be detected at 550 nm by an ELISA reader (680, BioRad, USA). The two solutions that make up this Griess reagent are (A); 0.1% W/V naphthylethylenediamine dihydrochloride in distilled water and (B); 1% W/V sulphanilamide in 3% V/V phosphoric acid and distilled water. Separately, the two solutions are generally more stable and have a longer shelf life; once combined, they cannot be stored for long due to auto-oxidation and must be utilized within 30 min. THP-1 cells were differentiated, as indicated in 2.4, and activated using 5 µl/mL of bacterial LPS in a 96-well plate (104 cells/well) for 72 h. 50 µl of the supernatant were combined with 100 µl of solution A and 100 µl of solution B were in a 96-microtiter plate and incubated at room temperature for 20 min in

dark, then absorbance at 550 nm was measured by a Multiskan bichromatic ELISA reader (680, BioRad, USA). The NO concentration was then calculated based on a standard curve and expressed in µM.

Determination of TNF-α, IL-6, and IL-10 production, induced by LPS

The anti-inflammatory properties of the FS extracts were analyzed using THP-1 cells. Cells were initially differentiated into macrophages in a 96-microtiter plate (10^4 cells/well), as mentioned in 2.4. After washing three times with PBS, cells were treated with 5 µg/mL LPS in the absence and presence of FS (0, 62.5, and 125 µg/mL). LPS-stimulated cells in the absence of plant extract were used as a positive control. TNF-α, IL-6, and IL-10 release were assessed using an ELISA kit (available from R&D Systems) in the supernatants of stimulated cells after 4 h, 6 h, and 24 h of treatment, according to the manufacturer's procedure. Cytokine levels were calculated based on a standard curve and expressed in pg/mL.

Statistical Analysis

Simple standard deviations of the mean are represented by the error limits given and the error bars shown. In most cases, numerical values are only reported to a level of precision that specifies the least significant digit. GraphPad PRISM 8 was used to perform multiple comparisons using one-way ANOVA followed by Dunnett's test. P 0.05 was used to define significance.

3. RESULTS AND DISCUSSION

Several scientific reports confirmed that psoriasis has an overexpression of pro-inflammatory type 1 cytokines, which is assumed to be pathophysiologically significant. [2, 5, 6]. Based on knowledge from traditional Greco-Arab and Islamic herbal medicine, FS is highly recommended in the management of various skin diseases. In this *in vitro* study, we evaluated the cytotoxicity, cytostatic, as well as anti-inflammatory mechanism of water/ethanolic extracts of FS leaves and fruits by measuring the production of both anti-inflammatory (IL-10) and pro-inflammatory (NO, IL-6, and TNF-α) mediators in the human keratinocyte cell line HaCaT and human THP-1-derived macrophages and their co-cultures. We applied a co-culture system of HaCaT and THP-1-derived macrophages to mimic the *in vivo* situation where both cell types contribute to the manifestation of psoriasis [2, 5, 6].

Cytotoxic and cytotoxic effects of FS extracts

Keratinocytes typically mature and shed from the skin every 30 days, however as psoriasis progresses, this maturation time shortens to three to six days and shifts to the cutaneous surface. Furthermore, in psoriasis, active keratinocytes drive immune cell infiltration [25]. Hence, possible method of controlling psoriatic lesions would be to normalize keratinocyte development or promote keratinocyte death. An ideal agent for treating psoriasis should inhibit keratinocyte proliferation as well as the inflammatory process.

In scientific and clinical research, the idea of employing pure phytochemicals and crude extracts derived from herbs as antiproliferative agents in the treatment of psoriasis has gained great interest recently[26, 27]. The downregulation of keratinocyte-hyperproliferation and modulation of keratinocyte apoptosis are promising potential psoriasis therapeutic targets for both approved medications and as well as herbal-derived remedies [28]. It may be advantageous to highlight the synergistic impact of treating diseases when crude extracts

with bioactive compounds produced from herbs are combined. It has been explored if crude extracts are effective in treating psoriasis hyperproliferation. In several traditional or folk remedies, the majority of these substances have been primarily used to treat inflammatory illnesses. Psoriasis is addressed using traditional Chinese medicine, which is widely used and has high safety and efficacy [29].

Here we utilized the MTT assay to examine FS extracts at increased concentrations on THP-1-derived macrophages, the HaCaT cell line, and their co-cultures after 24 h (cytotoxic effects) and 72 h (cytostatic effects). Figures 1 and 2 show the cytotoxic and cytostatic results of FS extracts in the three culture systems. FS extracts showed no significant cytotoxic effects in both monocultures and co-cultures. Significant cytostatic effects were seen after treatment of HaCaT cells with leaves and fruit extracts. No significant cytostatic effects were seen in cells from the THP-1 cell line. The antiproliferative effects in the co-cultures were at lower levels compared to HaCaT monocultures. The fruits extract exhibited higher inhibitory effects compared to the leaves extract as the IC_{50} values for leaves and fruits extracts were 911.5 and 656.6 µg/mL, respectively. The cytostatic effects were lower in the co-culture (IC_{50} 886.7 µg/mL) compared to the monoculture (IC_{50}= 656.6 µg/mL) for fruits extracts, as well as for leaves ones 2097 and 911.5 µg/mL, respectively (Table 2).

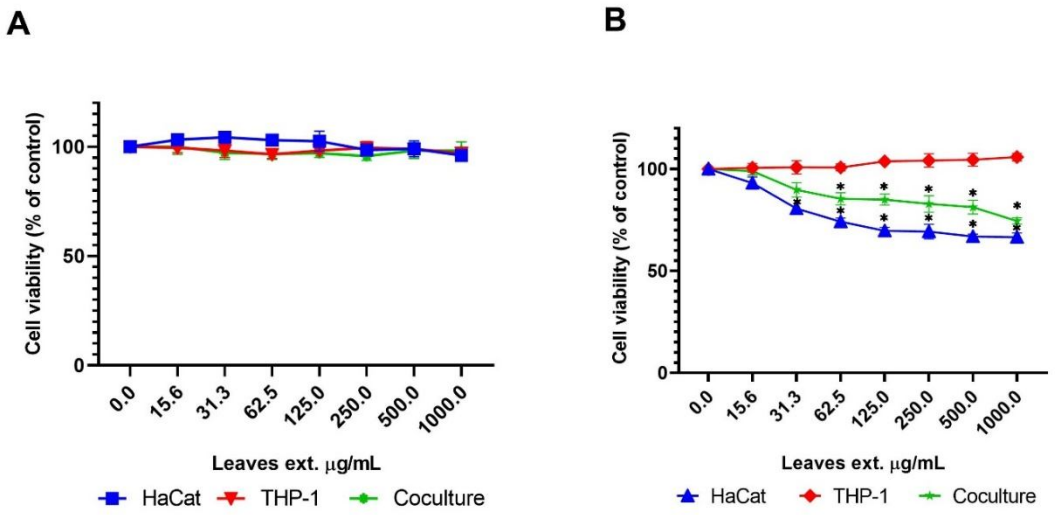

Fig. 1. MTT Assay in THP-1-derived macrophages, HaCaT cells, and their co-cultures after treatment with increased concentrations of FS leaves extract for (A) 24 h (cytotoxic effects), and (B) 72 h (cytostatic effects). In an ELISA reader, the MTT formazan's absorbance was measured at 570 nm. The absorbance ratio of cells treated with FS extracts in comparison to untreated cells was used to measure cell viability (reported as a percentage). Values represent means ± SD (*P< 0.05 was considered significant compared to control) of three independent experiments performed in triplicates.

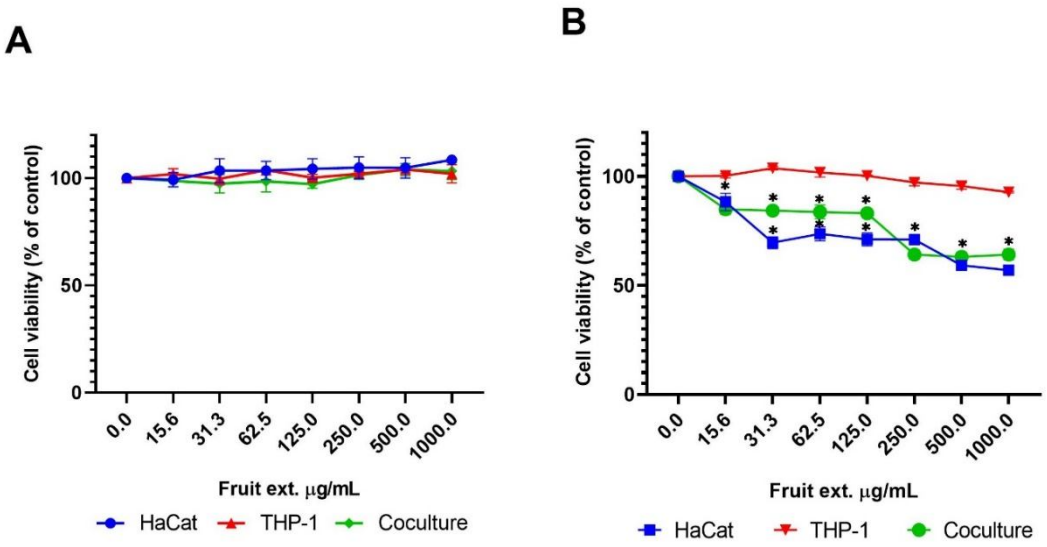

Fig. 2. MTT Assay in THP-1-derived macrophages, HaCaT cells, and their co-cultures after treatment with increased concentrations of FS fruit extracts for (A) 24 h (cytotoxic effects), and (B) 72 h (cytostatic effects). In an ELISA reader, the MTT formazan's absorbance was measured at 570 nm. The absorbance ratio of cells treated with FS extracts in comparison to untreated cells was used to measure cell viability (reported as a percentage). Values represent means ± SD (*P< 0.05 was considered significant compared to control) of three independent experiments performed in triplicates.

Table 2. IC50 values in µg/mL of the cytostatic effects of FS leaves and fruits extracts. N/D: Not determinable

Cell type	Cytostatic	
	Leaves	Fruits
HaCaT	911.5	656.6
THP-1	N/D	N/D
Co-cultures	2097	886.7

Our results agree with many other published studies that indicated FS extracts had cytostatic effects on a variety of cancer cells and also showed that these effects were influenced by the phenolic content of the extracts [30]. In their evaluation of the antioxidant, antimicrobial, and anticancer properties of FS fruit and leaf extracts, El-Beltagi et al. [30] found that the ethanolic and ethyl acetate extracts of FS leaves have higher concentrations of total phenols, flavonoids, tannins, alkaloids, and steroids than the fruit extracts. In contrast to ethyl acetate extracts, ethanol extracts of both fruits and leaves had higher concentrations of phytochemical components. Therefore, it was observed that fruit and leaf extracts had antioxidant and cytotoxic effects on the HepG2 liver cell line, the Caco-2 colorectal cancer cell line, and the MCF-7 breast cell line (MCF-7) [30]**.** In addition, studies have investigated the efficacy of crude extracts for treating psoriasis hyperproliferation *in vitro*

using keratinocyte cell lines cultured in monoculture systems. For example, Tse et al.[31] used HaCaT to assess the cytostatic effect of 60 Chinese antipsoriatic medicinal materials. They reported that the antiproliferative efficacy of *Coptis chinensis*, realgar and *Rubia cordifolia* were demonstrated by their IC50 values of 23.4, 6.6, and 1.4 g/mL respectively. Moreover, Tse et al. [32] further explored the molecular mechanisms of the antiproliferative activity against HaCaT and suggested that *Rubia cordifolia* is mediated through induction of apoptosis. Most of the published medicinal plants' antiproliferative effects on keratinocytes were based on monoculture systems. Here in our present study, we applied a co-culture system of HaCaT and THP-1-derived macrophages to better mimic the *in vivo* situation where both cell types contribute to the manifestation of psoriasis. As seen in Figure 1 and Figure 2 the antiproliferative effects of FS extracts are significantly different in both culture systems. Therefore, further experiments are needed to investigate the mechanism responsible for this observation.

Anti-inflammatory effects

Macrophages are responsive immune cells that can carry out various functional and transcriptional systems in response to microenvironmental factors, especially when exposed to microbial products that pose a risk to the body, through the production of cytokines and pro-inflammatory mediators such as NO synthesized by inducible nitric oxide synthase (iNOS) [33]. As they emit pro-inflammatory signals such as interleukins (IL-1 and IL-6), tumor necrosis factor (TNF-α), and other mediators, macrophage cells play a crucial role in the inflammatory process [33]. This process relies on the generation of inflammation by LPS in THP-1-derived macrophages, an abundant endotoxin in the outer membrane of the majority of Gram-negative bacteria with an important role during host-pathogen interaction. [34]. A childhood instance of acute monocytic leukemia (M5 subtype) arose to the spontaneously immortalized monocyte-like cell line known as human THP-1 [35]. THP-1 cells are powerful tools for assessing monocyte activity in both healthy conditions and inflammatory disorders [35]. THP-1 cells represent valuable tools for measuring monocyte function in both health and inflammatory diseases [36-38]. Numerous physical, chemical, or microbiological factors can trigger the inflammatory process, which further causes a chain reaction of cellular processes. The release of key enzymes and chemical mediators, which respond in various ways and can regulate these processes [39].

In this study, the inflammatory response was induced using lipopolysaccharide. The production levels of NO, TNF-α, IL-6 and IL-10 were measured on LPS-activated THP-1-derived macrophages in the absence and presence of FS leaves and fruits extracts. Our analysis goes on to examine the inflammatory profile of the dietary supplement-treated cells at doses of 125 and 250 g/mL based on viability and cytotoxicity assays. Since the initial treatment of the THP-1-derived macrophages using the FS extracts did not generate an inflammatory response (data not shown), we treated the cells with LPS and afterward applied the treatment with the FS extracts.

Suppression of nitric oxide (NO) production by macrophages

In the course of the inflammatory process, nitric oxide is one of the mediators that causes vasodilation and the consequent rise in blood flow [39]. It is assumed that NO production as well as inflammatory response increase significantly with LPS induction [40]. Figure 3 shows how both leaf and fruit extracts decrease the LPS-mediated release of NO in a dose-dependent manner. At a concentration of 250 g/mL, FS extracts reduced the release of NO by cultured THP-1 cells in a dose-dependent manner, bringing levels to those of untreated cells. The inhibitory effects of the fruit’s extracts were significantly stronger than the leaves extracts, NO release was inhibited from 65 µM to 19 µM and 16 µM after treatment with

leaves and fruits at 250 µg/mL, respectively. This obtained result could be referred to the high contents of total phenols, flavonoids, tannins, alkaloids, and steroids in ethanolic extracts of FS fruits than in the leaves ones [30]. Similar results were recorded with medicinal plants crude extracts and phytochemicals (e.g. flavonoids) [36-38]. Various flavonoids, including apigenin, luteolin, and quercetin, were reported to decrease NO generation using LPS/cytokine-treated macrophages or macrophage-like cell lines. Well according to the studies on the mechanisms, flavonoids had little effect on iNOS inhibition. They were shown to inhibit iNOS induction, which decreased NO release [35]. Similarly, *Hypericum triquetrifolium* extracts were reported to suppress the LPS-induced NO release through attenuation of the LPS-induced transcription of iNOS [36]. The action mechanism by which FS extracts inhibit the NO secretion needs to be evaluated in future experiments.

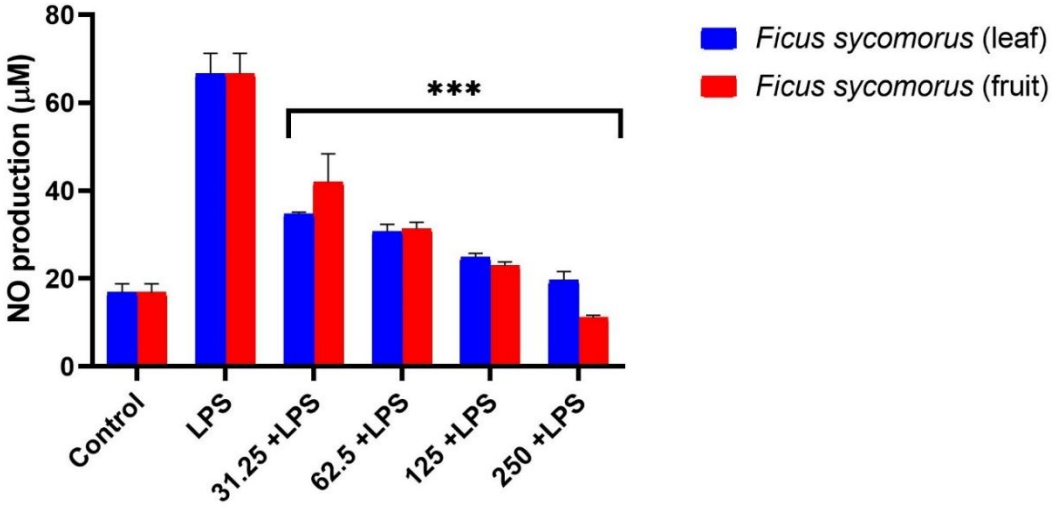

Fig. 3. NO production by THP-1-derived macrophages after treatment with of increased concentrations of leaves and fruit extracts of FS as measured with Griess assay. Values represent the mean of 3 independent experiments applied in triplicates. NO values of LPS treated cells were taken as 100%. * $p<0.05$, ** $p<0.01$, ***$p<0.001$ as considered significant compared to control group one-way ANOVA followd by Dunnett's test.

Suppression of pro-inflammatory cytokines IL-6 and TNF-α

Keratinocyte hyperproliferation in the basal and suprabasal epidermal regions, infiltration of hugely important inflammatory cells like macrophages, dendritic cells, neutrophils, lymphocytes, and T helper (TH) cells, and stressed keratinocytes that produce reactive oxygen and nitric oxide as well as pro-inflammatory cytokines and chemokine [5]. Production of the cytokines by THP-1-derived macrophages were examined in the culture supernatants using commercial ELISA kits. After being stimulated with LPS, macrophages generated from THP-1 release measurable levels of IL-6 (Figure 4) and TNF-α (Figure 5). The culture supernatants had maximum levels of TNF-α and IL-6 after 4 and 6 hours of LPS stimulation, respectively. Treatment of THP-1-derived macrophages with FS extracts alone were unaffected by the levels of any of the studied cytokines (data not shown). Figure 4 illustrates the release of IL-6 by untreated and LPS-activated THP-1-derived macrophages into the culture supernatant. The two pro-inflammatory cytokines' production was decreased by FS extracts in a dose-dependent manner. TNF-α and IL-6 secretion from LPS-activated THP-1 cells was about 709 pg/mL and 818 pg/mL, respectively. Nevertheless, when same activated cells were treated FS extracts at a concentration of 250 g/mL, TNF-α and IL-6 secretion

were significantly inhibited. The IL-6 (Figure 5) was reduced to 563 and 596 pg/mL after treatment with leaves and fruits extracts at 250 µg/mL, respectively. The inhibitory effect was stronger on TNF-α (Figure 4) secretion and reached 325 and 209 pg/mL after treatment with leaves and fruits extracts at 250 mg/mL, respectively. Similar findings were observed using several therapeutic herbs. For example, *Hypericum triquetrifolium* and *Peganum harmala* in THP-1 cells reduced NO and TNF-α production as well as iNOS and TNF-α expression, but not IL-6. These inhibitory effects of the pro-inflammatory cytokines and the elevation of the anti-inflammatory IL-10 was suggested to be the result of inhibiting their mRNA transcription [36, 38, 41, 42].

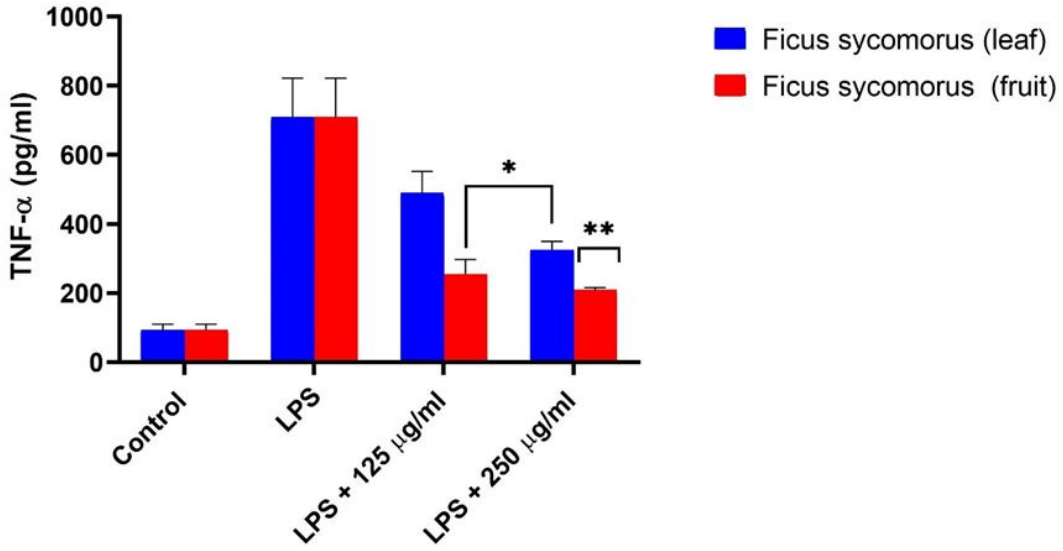

Fig. 4. TNF-α release by LPS-activated THP-1 cells. The cells were treated with increased concentrations ofleaves and fruit extracts of FS. TNF-a release was assessed using an ELISA kit after 4 h of treatment. The percentage of means and standard deviations are indicated by bars. Values represent means ± SD, (* $p<0.05$, ** $p<0.01$, ***$p<0.001$ was considered significant compared to control) of three independent experiments carried out in triplicates.

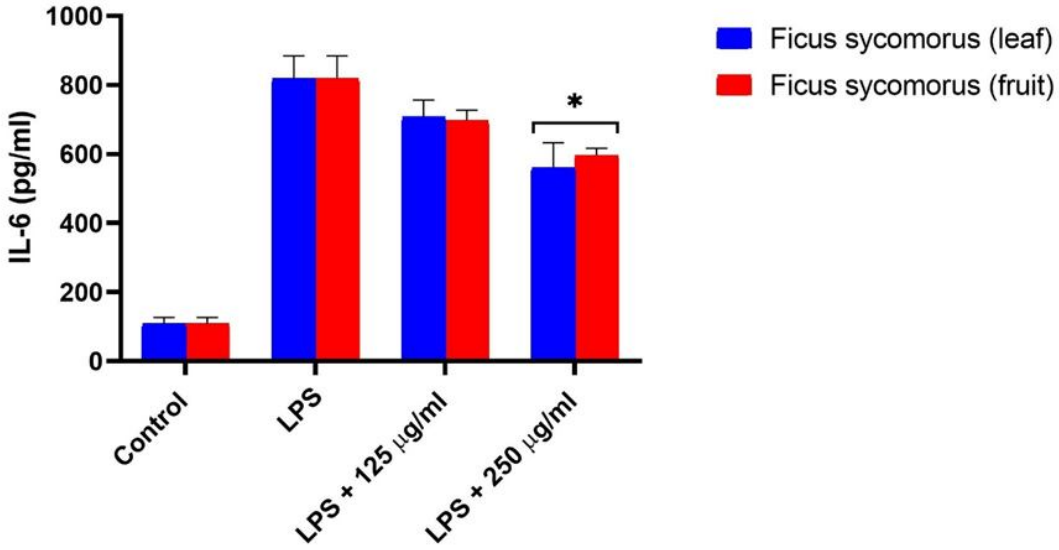

Fig. 5. IL-6 release by LPS-activated THP-1 cells. The cells were treated with increased concentrations of leaves and fruit extracts of FS. IL-6 release was assessed using an ELISA kit after 6 h of treatment. The percentage of means and standard deviations are indicated by bars. Values represent means ± SD, (* $p<0.05$, ** $p<0.01$, ***$p<0.001$ was considered significant compared to control) of three independent experiments carried out in triplicates.

Increasing of anti-inflammatory cytokine IL-10

A modest amount of the immunomodulatory cytokine was secreted by THP-1-derived macrophages after being exposed to LPS. IL-10. The highest IL-10 levels detected in the culture supernatants were 24 hours after LPS stimulation, reaching 74 pg/mL. However, IL-10 levels increased in the cells when FS extract was applied, with the maximum concentration being at 125 g/mL. (Figure 6). Similar findings for *Peganum harmala* and *Hypericum triquetrifolium* have also been published [36, 38, 41, 42].Both plants were found to increase levels of IL-10 protein and mRNA expression. When compared to the highest inhibitory concentrations of IL-6 and TNF-α, the effects of *Hypericum triquetrifolium* and *Peganum harmala* were seen at substantially lower extract concentrations for FS. A strong immunosuppressor cytokine is IL-10. It aids in the reduction and termination of inflammatory responses [43]. The suppression of LPS-induced IL-6 and TNF-α production by FS extract may occur through the stimulation of IL-10 secretion, according to prior studies that have demonstrated the antagonistic action of IL-10 on the secretion of pro-inflammatory cytokines [44, 45]. The two hallmarks of psoriasis and other inflammatory diseases are a very low blood level of IL-10 and a high blood level of TNF-α. Moreover, administration of the exogenous recombinant version of IL-10 resulted in lowering in blood levels of TNF-α, which has been shown to be beneficial for such disorders [46].

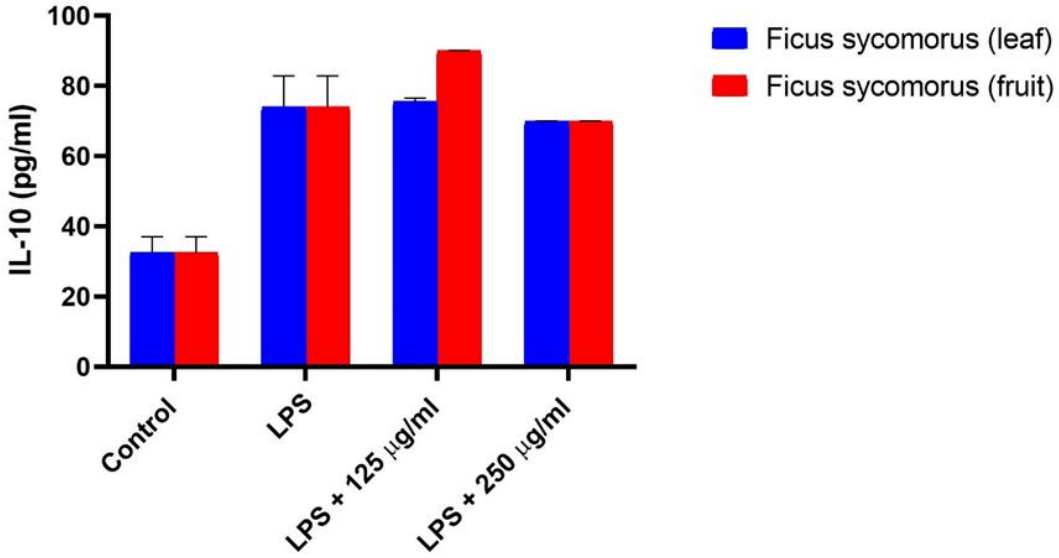

Fig. 6. IL-10 release by LPS-activated THP-1 cells. The cells were treated with increased concentrations of leaves and fruit extracts of FS. IL-10 release was assessed using an ELISA kit after 24 h of treatment. The percentage of means and standard deviations are indicated by bars. Values represent means ± SD, (* $p<0.05$, ** $p<0.01$, ***$p<0.001$ was considered significant compared to control) of three independent experiments carried out in triplicates.

4. CONCLUSION

The potential therapeutic application of FS extracts in psoriasis and other inflammation-related diseases was indicated by their ability to upregulate the anti-inflammatory cytokine and downregulate the pro-inflammatory cytokine. However, a more thorough investigation of the effects of FS extracts on these cytokines is required given that FS extracts alter the production of pro-inflammatory and anti-inflammatory cytokines at various concentrations.

CONSENT (WHERE EVER APPLICABLE)

It is not applicable.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

It is not applicable.

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