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Anti-inflammatory effect of *Solanum nigrum* Linn. ethanolic leaves extract for oral ulcer in LPS stimulated THP 1 cell line

Research Article

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Abstract

Background: Recurrent Aphthous Stomatitis (oral ulcer) is a frequent inflammatory disorder affecting the oral mucosa manifested as painful recurring ulcers that impair quality of life. Current treatments primarily involve corticosteroids, which have various adverse effects. *Solanum nigrum* Linn. is a widely used medicinal herb in India. Traditionally used in ayurveda, to treat liver disorders and balance the three doshas-*Vata, Kapha* and *Pitta*. Its anti-gastric ulcer effects have been reported in ethnopharmacological studies using experimental ulcer models. However, to the best of our knowledge, no studies have assessed its effects on inflammatory mediators involved in oral ulcers. This study aimed to investigate the anti-inflammatory effects of *Solanum nigrum* Linn. on inflammatory mediators of oral ulcer using the THP1 cell line, an in vitro model for inflammatory diseases. Materials and methods: Ethanolic extract of *Solanum nigrum* Linn. leaves was prepared using the Soxhlet apparatus and subjected to phytochemical screening. THP 1 cells were differentiated into macrophages using phorbol 12- myristate 13- acetate (PMA) and activated by lipopolysaccharide (LPS). An MTT assay was done to measure cell viability. Results: THP1 cells were pretreated with extract concentrations of 5, 10, 50, 100 µg/mL. Treatment with 100µg/mL of *Solanum nigrum* Linn. extract resulted in a statistically significant decrease in inflammatory mediators TNF-α, IL-1β, and IL-6 ($p<0.05$). Conclusion: *Solanum nigrum* Linn extract demonstrated significant anti-inflammatory activity by down regulating key inflammatory mediators involved in oral ulcer. These findings suggest that *Solanum nigrum* Linn. can be used as a potential therapeutic formulation for managing oral ulcer.

Keywords: Cell line**,** Corticosteroids, Herbal, Inflammation, Inflammatory mediators, Oral ulcer.

Introduction

The oral ulcer is not an uncommon oral mucosal disorder affecting nearly 25% of the global population (1). These ulcers, often round or oval with a greyish-yellow membrane surrounded by an erythematous halo, typically occur on the buccal mucosa, labial mucosa, and tongue (2). While its precise etiology remains elusive, contributing factors include genetic predisposition, immune dysfunction, nutritional deficits, microbial infections, food allergies, stress, hormonal changes, and mechanical trauma (3).

Although the pathogenesis of oral ulcer is not completely understood, evidence indicates the critical role of mediators involved in inflammation such as cytokines in the pathogenesis of oral ulcer. Interleukin-1β (IL-1β), Tumor Necrosis Factor-α (TNF-α), and Interleukin-6 (IL-6) are observed predominantly with elevated levels in oral ulcer lesions (4,5).

Multiple treatment modalities have been tried to control the inflammation and pain ranging from corticosteroids to immunosuppressants such as thalidomide. These treatment modalities possess significant side effects leading to drug resistance and adverse drug reactions, highlighting the need for alternative therapeutic strategies (5).

Plants are a substantial source of therapeutic phytochemicals and are safe, effective, and have few adverse effects. They produce secondary metabolites (SMoP's) such as terpenoids, alkaloids, nitrogen-containing compounds, organosulfur compounds, and phenolic compounds which exhibit bioactivities like anti-inflammatory, immunostimulatory, anticancer, antioxidant and antibacterial properties (6,7). In recent times, medicinal plants have been an important source of novel therapeutic agents with the potential for developing new treatments for oral ulcer.

One such plant is *Solanum nigrum* Linn. (*S. nigrum* L.) well-known as "Black nightshade" or "*Kakamachi*" in Ayurveda. It is greatly used in herbal medicine to manage conditions such as *Shotha* (inflammation), *Yakritshotha* (liver disorders) and *Vrana* (wounds) (8). The plant contains major active

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components such as glycoalkaloids, polysaccharides, glycoproteins, and polyphenolic compounds (9). A study by Padalia K in 2014 showed that the leaves and young stem of *S. nigrum* L. have been used by the tribal people of the central Himalayan region in the form of ointment as *Gewai saag* to relieve pain (10).

The anti-ulcer activity of *S. nigrum* L. extract has been demonstrated in various animal models of gastric ulcers. A study on gastrointestinal ulcers using *S. nigrum* L. fruit extract concluded that the extract has anti-ulcerogenic and gastric ulcer healing properties (11). The present study intends to decipher the impacts of *S. nigrum* L. on the common oral inflammatory disorder oral ulcer.

In THP 1 cell line monocyte is activated into macrophages by inducing Vitamin D3 or phorbol12-myristate 13-acetate [PMA] and as such they can be used to study the in vitro assessment of inhibition of commonly expressed oral ulcer inflammatory mediators TNF-α, IL-1β and IL-6 (4,12). With this background, the current study aims to assess the effect of *S. nigrum* L. ethanolic extract on inflammatory mediators of oral ulcer using the THP1 cell line which may be used as a promising treatment modality for oral ulcer.

Materials

Authentication of plant material and processing:

The plants were obtained from the local marketplace in Pondicherry, India. The plant was identified by a qualified botanist Professor R. Selvanathan, at the Centre de Phyto Botanicals Research (CPBR), Pondicherry, India.

Chemicals

Ethanol, dimethyl sulfoxide, gallic acid, Folin–Ciocalteu reagent, lipopolysaccharide (LPS), phosphate buffered saline, trypan blue, Mayer's reagent, ferric chloride, ammonia, chloroform, sulfuric acid, methanol, quercetin, aluminium chloride, sodium bicarbonate, sodium hydroxide, dragendorff's reagent, bromocresol green were procured from Sigma- Aldrich, Bangalore, Karnataka, India. The National Centre for Cell Science (NCCS) in Pune, India, is from which the THP 1 cell line was purchased.

Preparation of extract

The collected fresh leaves were cleaned well to eliminate any of the remaining soil materials. The leaves were then dried in the shade and coarsely powdered. The dried fine leaf powder was kept in the Soxhlet apparatus thimble chamber. Based on previous studies ethanol was used as a solvent for extraction (13) and the ethanolic extract was used for preliminary phytochemical screening. The extraction solvent was heated in the round-bottom flask, dissipated into the thimble container, cooled in the condensation chamber, and dripped down. The extraction technique involved weighing approximately 100 g of dry sample powder and using 250 mL of ethanol in the Soxhlet equipment for 2 days. The concentrated extract was dried by evaporation at 55 °C for 1 hour.

Evaluation of phytochemical screening

An initial phytochemical analysis was conducted on *S. nigrum* L. leaves to determine the presence of sterols, saponins, quinones, flavonoids, carbohydrates, alkaloids, tannins, terpenoids, phenol, and phlobatannins following the procedures outlined by Kokate *et al*., 1996.

Quantitative estimation of phenol, flavonoid, alkaloid:

Total alkaloid Estimation

Ten millilitres of weighed plant extract residue were diluted with ten millilitres of 2N HCL and then filtered. A separating funnel was filled with this solution (5 mL), and it underwent three rinses with 10 mL of chloroform each time. The solution pH was neutralized by adding 0.1N sodium hydroxide. The solution was thereafter combined with five millilitres of bromocresol green solution and phosphate buffer. The complex was extracted from the mixture using vigorous agitation, stored in a 10-milliliter conical flask, and neutralized by five millilitres of chloroform. After filling various separatory funnels, the atropine standard solution was extracted using five millilitres of chloroform. In UV Spectrophotometer at 470 nm in the spectrum, the complex's absorbance in chloroform was determined.

Total flavonoid estimation

The mixture was then placed at ambient temperature for an hour to sediment. The reactant mixture's absorbency was measured using a UV spectrophotometer at 420 nm and compared to a blank. The calibration plot was utilized to ascertain the whole flavonoid content concentration in test samples, with quercetin serving as the standardization material. The stock quercetin mix was made by adding one milligram of quercetin in one millilitre of methanol. The standard quercetin solution (0-1000 µg/mL) was created by diluting the stock solution with methanol.

Total Phenol estimation

The Folin-Ciocalteu reagent was diluted 1:10 into the plant extract to determine its total phenolic content. The standard was used as a guide to create the gallic acid calibration curve. A 0.5 mL extract solution (1000 g/mL) was mixed with Folin-Ciocalteu reagent (1 N) (2000 mL). After three minutes of stillness, the reactant mixture was allowed to stand at ambient temperature for thirty minutes, with intermittent shaking to check for colour development. 2% sodium carbonate (2 mL) was added. A UV spectrophotometer was then used to measure absorbance at 750 nm. The results were expressed as gallic acid equivalents (mg GAE/g) per gram of extract. A standard curve was developed using gallic acid concentrations ranging between 0 to 100 µg/ml.

THP-1 Cell Culture and Macrophage Differentiation

The cells of THP-1 were grown in RPMI-1640 media containing fetal bovine serum and antibiotics. Phorbol 12-myristate 13 acetate (PMA) was used to stimulate macrophage differentiation, which was then activated by 100 ng/mL of LPS (Sigma-Aldrich, Bangalore).

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Analysis of cell viability (MTT assay)

MTT assay was used to evaluate cell viability. The cells of THP-1 were subjected to varying doses of crude extract (5–100 µg/mL). In brief, 50 microlitre/ well serum-free media and 50 microlitre /well MTT reagents were used in place of the media. To cultivate the cells 24-well plates were used and incubated for three hours. A microplate reader (Spectra Max) was used to measure the optical density (OD) at 590 nm. Each assessment was carried out at least three times.

Enzyme-linked immunosorbent assay (ELISA)

In this experiment, bacterial LPS was used to activate 4 × 10^4 THP-1 cells that had been planted into each well of a 96-well plate. After being pre-treated with PMA (4 × 10^4), the cells were cultivated for 48 hours in a 96-well plate in two different conditions: 100 ng/mL LPS extract only and LPS (100 ng/mL) alone. Centrifuging at 1000 rpm for 8 minutes at 4°C yielded the supernatant after 48 hours, which was then kept at -20°C. ELISA kits were used to quantify the levels of TNF-α and IL. As positive and negative controls respectively, DMSO and LPS were utilized at a final dose of 1 µg/mL.

The wells were filled with samples that were diluted and standardized, and they were incubated for ninety minutes at 37°C. After washing the plates, detection antibodies were incorporated, and they were then incubated for an hour at 37°C. The Avidin-Biotin-Peroxidase Complex was introduced to the plates following further washing, and they were then incubated at 37°C for 30 minutes. After 15 to 25 minutes of culture at 37°C, a stop solution was administered and a color-developing reagent was added. At 450 nm, the optical densities were measured. The levels of TNF-α, IL-6, and IL-1β were expressed in pg/mL, and each experiment was carried out in triplicate.

Statistical analysis

Data were examined using one-way ANOVA and Tukey's test to evaluate statistical significance, with $p < 0.05$ considered significant.

Results

Phytochemical screening

On preliminary phytochemical screening of *S. nigrum* L. leaf, quinones, sterols, alkaloids, glycosides, flavonoids, phenol, tannins, and terpenoids were present in ethanolic extract.

Estimation of total alkaloid content

Using the linear regression, the total alkaloid content in *S. nigrum* L. leaves was found to be 112 µg/ mL (as shown in Figure 1, 4). This indicates a significant alkaloid content in the leaves of *S. nigrum* L.

Estimation of total phenolic content:

Using the gallic acid standard curve, the total phenolic content in *S. nigrum* L. leaves was found to be 64 µg/mL (as shown in Figure 3, 4). This indicates that *S. nigrum* L. leaves contain a significant phenolic content.

Estimation of total flavonoid content:

Using the linear regression, the total flavonoid content in *S. nigrum* L. leaves was found to be 633 µg/ mL (as shown in Figure 2, 4). This indicates a significant flavonoid content in the leaves of *S. nigrum* L.

Figure 1: Estimation of total alkaloid content

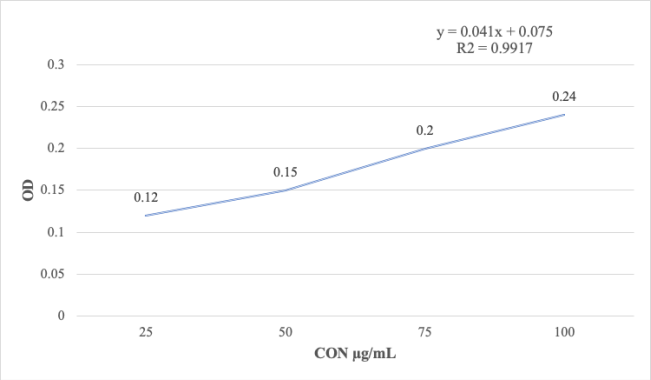


Figure 2: Estimation of total flavonoid content

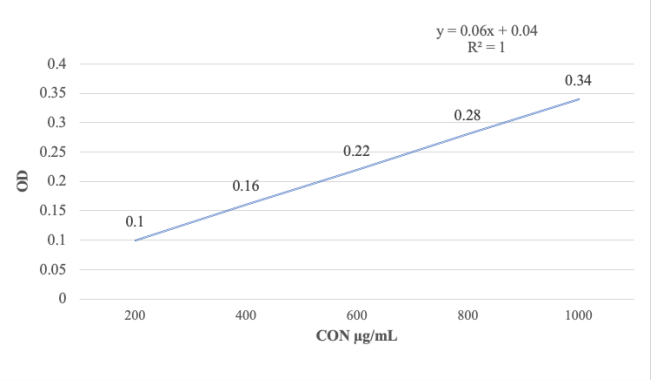


Figure 3: Estimation of total phenolic content

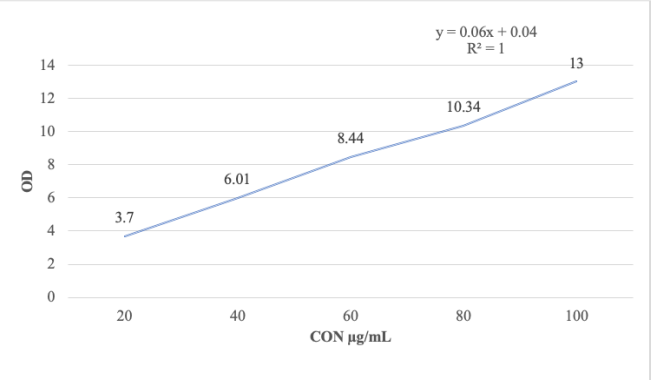
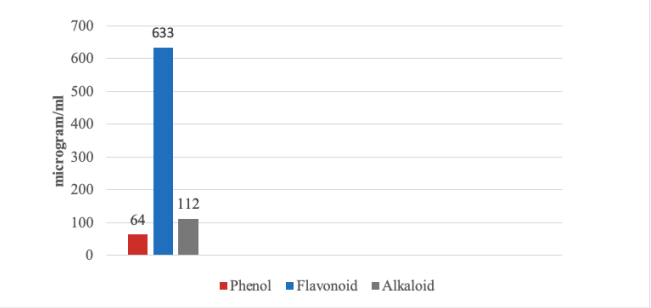


Figure 4 Quantitative estimation of phytochemicals



In vitro cytotoxicity of *S. nigrum* L.

The untreated group has 100% viability, while the control (DMSO- dimethyl sulfoxide) shows a slight decrease in viability over time. The treatment groups (5 to 100 µg/mL) exhibit a clear dose-dependent and time-dependent reduction in viability, highlighting the cytotoxic effects of the treatment. The ethanolic extract exhibited marked cytotoxicity at high concentration (100 µg/mL) in 96 hours as shown in Table 1 & Figure 5.

Figure 5: Various concentrations exhibited different viability at different times

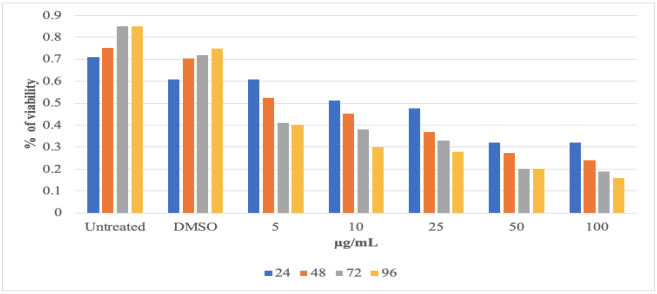


Table 1: In vitro cytotoxicity of *S. nigrum* L.

S.No.	Extract	Mean ± Standard
1	5	0.4 ± 0.008
2	10	0.3 ± 0.01
3	25	0.2 ± 0.009
4	50	0.2 ± 0.009
5	100	0.1 ± 0.009

Effect of S. *nigrum* L. extract on TNF α production stimulated by LPS

The mean value of TNF- α measured at control (350± 2.0), LPS (1280± 3.6), S5 (402±1.0), S10 (320), S50 (337±1.0), S100 (320± 6.0). The ANOVA test showed that there were a significant ($p< 0.05$) differences in TNF-α levels among the different concentrations as shown in Table 2 & Figure 6.

Figure 6: Levels of TNF - α at various concentrations

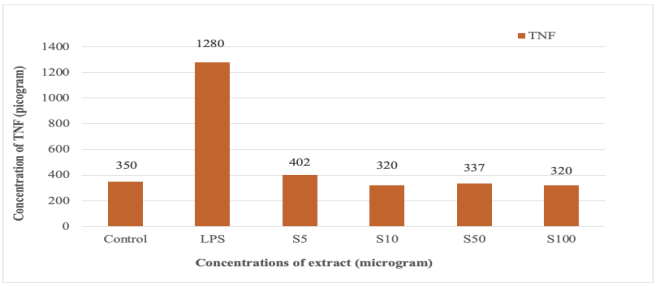


Table 2: Comparison of TNF - α levels (pg/mL) across different treatment groups, including control, LPS, and varying concentrations of treatment "S" (5 µg to 100 µg)

Mediators	Groups (µg)	Number of tests	Mean ± Standard deviation	p value
TNF - α	Control	3	350.0 ± 2.0	0.001
	LPS	3	1280.0 ± 3.6	
	S5	3	402.0 ± 1.0	
	S10	3	320.0 ± 0.0	
	S50	3	337.0 ± 1.0	
	S100	3	320.0 ± 6.0	
	Total	18	501.5 ± 359.4	

Effect of *S. nigrum* L. extract on IL-1 production stimulated by LPS

The mean value of IL - 1β measured at control (158± 3.0), LPS (650± 3.0), S5 (450.3±0.6), S10 (340± 6.0), S50 (304±4.4), S100 (220± 1.4). The ANOVA test showed that there were significant ($p< 0.05$) differences in IL-1β levels among the different concentrations as shown in Table 3 & Figure 7.

Figure 7: Levels of IL - 1β at various concentrations

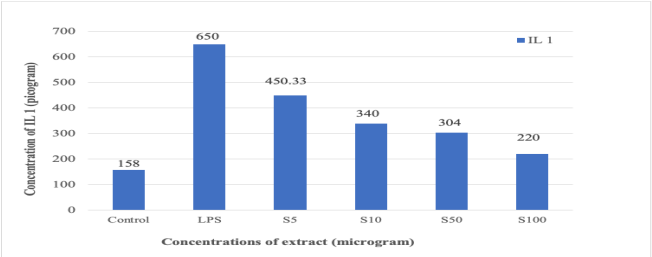


Table 3: Comparison of IL- 1β levels (pg/mL) across different treatment groups, including control, LPS, and varying concentrations of treatment "S" (5 µg to 100 µg).

Mediators	Groups (µg)	Number of tests	Mean ± Standard deviation	p value
IL -1β	Control	3	158.0 ± 3.0	0.001
	LPS	3	650.0 ± 3.0	
	S5	3	450.3 ± 0.6	
	S10	3	340.0 ± 6.0	
	S50	3	304.0 ± 4.4	
	S100	3	220.0 ± 1.4	
	Total	18	353.7 ± 165.9	

Effect of *S. nigrum* L. extract on IL-6 production stimulated by LPS

The mean value of IL - 6 measured at control (170.3± 2.5), LPS (750± 4.0), S5 (520±2.0), S10 (460± 5.0), S50 (340±3.0), S100 (230). The ANOVA test showed that there were significant ($p< 0.05$) differences in IL 6 levels among the different concentrations as shown in Table 4 & Figure 8.

Figure 8: Levels of IL - 6 at various concentrations

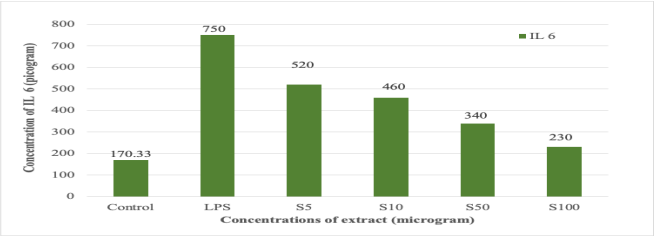


Table 4: Comparison of IL- 6 levels (pg/mL) across different treatment groups, including control, LPS, and varying concentrations of treatment "S" (5 µg to 100 µg)

Mediators	Groups (µg)	Number of tests	Mean ± Standard deviation	p value
IL- 6	Control	3	170.3 ± 2.5	0.001
	LPS	3	750.0 ± 4.0	
	S5	3	520.0 ± 2.0	
	S10	3	460.0 ± 5.0	
	S50	3	340.0 ± 3.0	
	S100	3	230.0 ± 0.0	
	Total	18	411.7 ± 199.3	

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Effect of *S. nigrum* L. extract concentrations on inflammatory mediators

The data were analyzed using Tukey's multiple comparisons test to assess the statistically significant differences in inflammatory markers of TNF- α, IL - 1β, and IL – 6 levels between the treatment groups of control, LPS, S5 µg, S10 µg, S50 µg, and S100 µg.

TNF - α - All group comparisons exhibit statistically significant differences (p = 0.01) except for the comparison between "S10 µg" and "S100 µg" groups, which shows no significant difference (p = 1.0) as shown in Table 5.

Table 5: Multiple comparisons of TNF - α levels between different treatment groups

Dependent Variable	(I) Groups (µg)	(J) Groups (µg)	Mean ± Standard deviation	p value
TNF – α	Control	LPS	-930.0 ± 2.5	0.001
		S5	-52.0 ± 2.5	
		S10	30.0 ± 2.5	
		S50	13.0 ± 2.5	
		S100	30.0 ± 2.5	
	LPS	S5	878.0 ± 2.5	0.001
		S10	960.0 ± 2.5	
		S50	943.0 ± 2.5	
		S100	960.0 ± 2.5	
	S5	S10	82.0 ± 2.5	0.001
		S50	65.0 ± 2.5	
		S100	82.0 ± 2.5	
	S10	S50	-17.0 ± 2.5	0.001
		S100	0.0 ± 2.5	0.99
	S50	S100	17.0 ± 2.5	0.001

IL - 1- Statistically significant differences (p = 0.01) are observed across all group comparisons as shown in Table 6

Table 6: Multiple comparisons of IL -1β levels between different treatment groups

Dependent Variable	(I) Groups (µg)	(J) Groups (µg)	Mean ± Standard deviation	p value
IL - 1β	Control	LPS	-492.0 ± 2.9	0.001
		S5	-292.3 ± 2.9	
		S10	-182.0 ± 2.9	
		S50	-146.0 ± 2.9	
		S100	-62.0 ± 2.9	
	LPS	S5	199.7 ± 2.9	0.001
		S10	310.0 ± 2.9	
		S50	346.0 ± 2.9	
		S100	430.0 ± 2.9	
	S5	S10	110.3 ± 2.9	0.001
		S50	146.3 ± 2.9	
		S100	230.3 ± 2.9	
	S10	S50	36.0 ± 2.9	0.001
		S100	120.0 ± 2.9	
	S50	S100	84.0 ± 2.9	0.001

IL - 6 - Statistically significant differences (p = 0.01) are observed across all group comparisons as shown in Table 7.

Table 7: Multiple comparisons of IL-6 levels between different treatment groups

Dependent Variable	(I) Groups (µg)	(J) Groups (µg)	Mean ± Standard deviation	p value
IL – 6	Control	LPS	-579.7 ± 2.6	0.001
		S5	-349.7 ± 2.6	
		S10	-289.7 ± 2.6	
		S50	-169.7 ± 2.6	
		S100	-59.7 ± 2.6	
	LPS	S5	230.0 ± 2.6	0.001
		S10	290.0 ± 2.6	
		S50	410.0 ± 2.6	
		S100	520.0 ± 2.6	
	S5	S10	60.0 ± 2.6	0.001
		S50	180.0 ± 2.6	
		S100	290.0 ± 2.6	
	S10	S50	120.0 ± 2.6	0.001
		S100	230.0 ± 2.6	
	S50	S100	110.0 ± 2.6	0.001

Discussion

The oral ulcer is a widely prevalent inflammatory mucosal condition causing severe pain, and burning sensation. It's etiology remains unknown with non-specific triggers making its treatment difficult. In allopathy, corticosteroids remain the mainstay of treatment with the minor form of oral ulcer treated with topical corticosteroids while the major form of oral ulcer is treated with systemic corticosteroids (14). However, long-term and frequent exposure to corticosteroids is associated with well-known adverse effects such as oral candidiasis, mucosal atrophy, and increased susceptibility to infections. Due to these concerns, there has been a paradigm shift towards herbal treatments owing to their safety and minimal side effects (15).

The current study is a pioneering study that compares inflammatory cytokine levels of TNF-α, IL-6, and IL-1β in the THP1 cell line to previous studies.

This study assessed the inflammatory mediators TNF-α, IL-1β, and IL-6 which have been proven as reliable diagnostic markers for oral ulcer (4), to determine the phytochemical composition and anti-inflammatory efficacy of *S. nigrum* L. leaf ethanolic extract using THP 1 cell line. The quinones, sterols, alkaloids, glycosides, flavonoids, phenol, tannins, and terpenoids were identified by initial phytochemical screening.

Notably, significant alkaloid (112 µg/mL), flavonoid (633 µg/mL), and phenolic (64 µg/mL) content were found in the current study suggesting the anti-inflammatory properties of *S. nigrum* L. extract.

THP 1 cell line is a well-recognized marker to study inflammatory changes. Based on previous

literature, in this study the cell line of THP-1 was utilized to assess the anti-inflammatory impact of *S. nigrum* L. extract. (16) (17).

Bazrafshani *et al*.,2002 demonstrated the relationship between oral ulcer and known IL-1A, IL-1RN, IL-6, and IL-1B gene variants by genotyping 91 oral ulcer patients and healthy volunteers. The study found a substantial correlation between oral ulcer and the alleles of IL-1β and IL-6 (18).

Surboyo et al.,2022 carried out a comprehensive review, selecting 30 relevant studies to examine the variations in TNF-α levels in blood, saliva, and lesions between oral ulcer patients and healthy people. The TNF-α values in individuals who were healthy were considerably lower than those in oral ulcer participants, demonstrating that TNF-α levels can serve as a reliable diagnostic indicator for oral ulcer (19).

Xiao *et al*.,2022 evaluated cytokines found in oral ulcer patients' peripheral blood that can be applied as a diagnostic and treatment indicator for oral ulcer through a systematic review. A total of sixteen relevant case-control studies had been collected and compiled. TNF-α and IL-6 were the predominant mediators in the peripheral blood among oral ulcer participants, according to the bubble chart analysis (4).

Experimental research by Jainu *et al*., 2005 revealed that the oral administration of *S. nigrum* L. fruit extract on gastric ulcer-induced albino rat models displayed a statistically significant reduction in ulcer size (p-value <0.001) without any apparent toxicological effects, indicating its potential as an anti-ulcer agent (11). Kang *et al*., 2011 observed that the chloroform fraction of *S. nigrum* L. extract (SNE) significantly decreased IL-6 and TNF-α levels in macrophages (p-value < 0.05), indicating its ability as an anti-inflammatory agent (20). An experimental study conducted by Yeom *et al*., 2019 for SNE anti-inflammatory effect on acute ear edema in mice exhibited a strong anti-inflammatory effect by inhibiting nitric oxide production and reducing ear edema with statistically significant reduction (p-value <0.05) (21). Experimental research by Drakshayani N Benni *et al*., 2024 revealed that the oral administration of *Kakamachi* (*S. nigrum* L.) swarasa on carrageenan-induced inflammation in albino rat models displayed a statistically significant reduction in paw volume in the trial group (p-value <0.01) and a highly significant reduction in the standard group (p-value <0.001), indicating its potential as an anti-inflammatory agent comparable to indomethacin (22).

Based on the findings of the previously stated investigations, the current study assessed the anti-inflammatory properties of *S. nigrum* L. leaf ethanolic extract by evaluating its impact on inflammatory mediators such as TNF-α, IL-6, and IL-1. After stimulation with LPS, TNF-α levels were substantially greater (1280 ± 3.6 pg/mL) than in the control group (350 ± 2.0 pg/mL). Treatment with the extract at varying doses (5 to 100 µg/mL) reduced TNF-α levels dose-dependently, with the highest dose (100 µg/mL) reducing it to 320 ± 6.0 pg/mL as shown in Table 2. This implies that ethanolic extract of *S. nigrum* L. may effectively lower the production of TNF-α, which is essential for inflammatory reactions.

Similarly, IL-1 and IL-6 levels, elevated in the LPS-stimulated group (650 ± 3.0 pg/mL and 750 ± 4.0 pg/mL), dropped to 220 ± 1.4 pg/mL and 230 ± 0.0 pg/mL at the highest extract concentration (100 µg/mL) as shown in Tables 3 & 4. Multiple comparison tests confirmed significant differences between groups, supporting the extract's strong anti-inflammatory effects. The study demonstrated a statistically significant ($p = 0.01$) reduction in TNF-α, IL-6, and IL-1 at 100 µg/mL, confirming the anti-inflammatory and anti-ulcerative potential of *S. nigrum L.*, consistent with previous findings.

Based on the observations of this current cell line study that assessed the anti-inflammatory effect of *S. nigrum* L. ethanolic extract the preliminary observations indicate that it can be used as an alternative to allopathic treatment of oral ulcers. Its phenolic and flavonoid content, known for their anti-inflammatory properties, may contribute to the regulation of cytokine production, thereby mitigating inflammation and promoting healing in oral ulcer patients.

Conclusion

The observations of the present study exhibited substantial anti-inflammatory effects of *S. nigrum*. L against inflammatory mediators of oral ulcers which are known to be reliable diagnostic markers. This highlights its potential as an alternative therapeutic option for managing oral ulcer. It could serve as the basis for future therapeutic formulations, such as mouthwashes or oral gels. However, clinical trials and in vivo studies are necessary to confirm these effects in patients.

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