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Original Research Article

Comparative *in vitro* evaluation of anti-inflammatory properties of selenium disulfide and ketoconazole-based topical formulations

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ABSTRACT

Background: Seborrheic dermatitis and dandruff are chronic inflammatory scalp disorders associated with cytokine-mediated immune responses and impaired barrier function.
Methods: This *in vitro* study compared the anti- inflammatory efficacy of two topical formulations—Selsun S (1% selenium disulfide + 3% salicylic acid) and a comparator (2% ketoconazole + 2% salicylic acid)—using THP-1 monocytic cells. Both formulations significantly suppressed TNF-α production (IC_{50} <0.03 mg/ml), with the comparator (2% ketoconazole + 2% salicylic acid) exhibiting higher cytotoxicity.
Results: Selsun S demonstrated greater potency in reducing IL-6 (IC_{50}: 0.033 mg/ml versus 0.041 mg/ml) and IL-8 (IC_{50}: 0.032 mg/ml versus 0.045 mg/ml), likely due to enhanced skin penetration from its higher salicylic acid content.
Conclusions: These findings support Selsun S's superior anti-inflammatory activity and tolerability, indicating its potential utility in managing inflammatory scalp conditions.

Keywords: Selenium disulfide, Ketoconazole, Salicylic acid, TNF-alpha, IL-6, IL-8, MTT assay, Anti-inflammatory

INTRODUCTION

Chronic scalp conditions such as seborrheic dermatitis and dandruff are prevalent dermatological disorders characterized by erythema, flaking, and persistent irritation.[1] These conditions are primarily driven by an interplay of excessive sebum production, microbial colonization (notably by *Malassezia* species), and a heightened inflammatory response.[2,3] While the fungal etiology is well-documented, recent insights underscore the pivotal role of pro-inflammatory cytokines in sustaining the chronic nature of these scalps disorders.[4]

Topical antifungal agents, particularly selenium sulfide and ketoconazole, are widely prescribed as first-line therapies due to their ability to suppress fungal proliferation.[5] Selenium sulfide functions by reducing epidermal turnover and exhibiting cytostatic effects on the epidermis and follicular epithelium.[6] Ketoconazole, an imidazole antifungal agent, disrupts fungal cell membrane synthesis by inhibiting ergosterol production.[7] Both agents are frequently combined with salicylic acid, a beta-hydroxy acid with potent keratolytic and comedolytic properties, which facilitates desquamation and enhances the penetration of active compounds through the stratum corneum.[8]

Despite their extensive use in clinical and over-the-counter preparations, there is limited comparative data assessing the anti-inflammatory performance of these formulations beyond their antifungal activity.[9] The present in vitro study was designed to bridge this knowledge gap by evaluating and comparing the immunomodulatory potential of two marketed formulations: Selsun S (containing 1% selenium

disulphide + 3% salicylic acid) and comparator (containing 2% ketoconazole + 2% salicylic acid). Using THP-1 monocytic cells stimulated with lipopolysaccharide (LPS) to mimic an inflammatory environment, the study assessed the secretion levels of pro- inflammatory cytokines TNF-α, IL-6, and IL-8—following treatment with graded concentrations of each formulation.[10] Cell viability was concurrently evaluated using the MTT assay to determine cytotoxic thresholds. The resulting IC_{50} values and cytokine inhibition profiles offer insights into the relative potential of each formulation as an anti-inflammatory agent, with implications for their therapeutic roles in managing inflammatory scalp conditions.[11]

METHODS

Study design

This was an *in vitro* experimental study designed to evaluate and compare the cytotoxic and anti-inflammatory activities of two topical antifungal formulations. The study was conducted at KET's Scientific Research Centre, Animal Biotechnology and Biochemistry Division, Mumbai, India, in collaboration with Abbott Healthcare Pvt. Ltd., Medical Affairs Division, and Mumbai, India. The experimental work was carried out during the period September 2024 till January 2025.

Sample preparation

Two formulations provided by Abbott Healthcare Pvt. Ltd. were evaluated in this study: Selsun S (1% selenium disulphide + 3% salicylic acid), coded SRC/AB/24/01/01, and comparator (2% ketoconazole + 2% salicylic acid), coded SRC/AB/24/01/02.

Both samples were solubilized in RPMI 1640 medium, followed by centrifugation. The supernatant was collected and used for all downstream analyses. Based on experiments, concentration ranges were finalized for toxicity (0.015–0.62 mg/ml) and anti-inflammatory assays (0.03–0.5 mg/ml).[12]

Cell culture and differentiation

The human monocytic cell line THP-1 was used for cytotoxicity and immunochemical analyses.[13] Cells were seeded at a density of 0.5×10^4 cells/well in a 96-well plate, and differentiation was induced by adding 10 µl of 10 ng/ml PMA (phorbol 12-myristate 13-acetate). The plate was incubated at 37°C with 5% CO_2 for 48 hours to allow for macrophage-like differentiation.[14]

Cytotoxicity assay (MTT assay)

Following differentiation, the medium was discarded, and 100 µl of various concentrations of test samples and standard with and without lipopolysaccharide (LPS) were added to the wells. Cells were incubated for an additional 24 hours at 37°C/5% CO_2. After incubation, MTT assay was performed by adding 10 µl of MTT reagent (5 mg/ml) to each well, followed by incubation for 4 hours. The formazan crystals formed were solubilized using DMSO, and absorbance was measured at 570 nm using a microplate reader.[15] Percent viability was calculated using the following formula, where, t=absorbance of test sample and c=absorbance of untreated control.[16]

$$Percent\ viability = t/c \times 100$$

Immunochemical assays

To assess the anti-inflammatory activity of the test samples, the concentrations of pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8) were measured in the culture supernatants. Quantification was performed using enzyme-linked immunosorbent assay (ELISA) kits specific for each cytokine, procured from Krishgen Biosystems.[17] All procedures were carried out strictly in accordance with the manufacturer's protocols, including sample preparation, reagent addition, incubation times, and absorbance measurements. Cytokine levels were determined by comparing absorbance values to a standard curve generated using known concentrations of recombinant cytokines provided with the kits.

Percent inhibition of cytokine production was calculated as follows.

$$Percent\ inhibitory\ activity = (absorbance\ of\ LPS\ control - absorbance\ of\ sample)/(absorbance\ of\ LPS\ control)$$

Curves were generated to determine the IC_{50} values the concentration required to inhibit 50% of cytokine release by plotting percent inhibition versus concentration using non-linear regression analysis.[18,19]

Ethical approval

As this was an *in vitro* study using a commercially available human cell line, no direct involvement of human participants or animals was involved. Therefore, formal ethical committee approval was not required. However, all experimental procedures were conducted in accordance with institutional biosafety and laboratory guideline.[20]

RESULTS

Cytotoxicity analysis of Selsun S and comparator

The cytotoxic effects of Selsun S were evaluated on THP-1 cells using the MTT assay. A dose-dependent decrease in cell viability was observed across the tested concentrations (0.015–0.62 mg/ml) (Tables 1 and 2). At the highest concentration of 0.62 mg/ml, the average optical density (O.D.) was 0.070 (±0.001), corresponding to 26.52% cell viability, indicating substantial

cytotoxicity. The viability progressively increased with decreasing concentrations, reaching 72.01% at 0.015 mg/ml. The IC_{50} (half-maximal inhibitory concentration) was calculated to be 0.14 mg/ml, indicating moderate cytotoxic potential at concentrations exceeding this threshold. Moreover, Comparator demonstrated a similar trend in cytotoxicity, with a dose-dependent reduction in viability. At 0.62 mg/ml, the average O.D. was 0.066 (±0.009), correlating to 25.00% viability. A gradual increase in cell viability was seen at lower concentrations, peaking at 80.56% at 0.015 mg/ml. The IC_{50} was calculated at 0.12 mg/ml, suggesting higher cytotoxic potency compared to Selsun S.

TNF-alpha inhibitory activity of Selsun S and comparator

Selsun S significantly inhibited TNF-α secretion in LPS-stimulated THP-1 cells in a concentration- dependent manner (Table 3). At 0.5 mg/ml, the average O.D. was 1.398 (±0.01), equating to 93.67% inhibition of TNF-α. Even at the lowest tested concentration (0.03 mg/ml), substantial inhibition (67.27%) was observed. The IC50 was determined to be below 0.03 mg/ml, demonstrating potent anti- inflammatory activity. Comparator also demonstrated strong TNF-α inhibition (Table 4).

At 0.5 mg/ml, inhibition was 87.47%, with the lowest dose (0.03 mg/ml) still showing 75.31% inhibition. Like Selsun S, the IC50 was also determined to be below 0.03 mg/ml, indicating high anti-inflammatory response in suppressing TNF-α production.

IL-8 inhibitory activity of Selsun S and comparator

Selsun S markedly inhibited IL-8 secretion in a dose-responsive manner (Table 5). Maximum inhibition was observed at 0.5 mg/ml with an average O.D. of 4.520 (±0.06), translating to 99.82% inhibition. The inhibitory effect declined with decreasing concentrations, yet remained notable, with 36.99% inhibition at 0.03 mg/ml. The IC_{50} was calculated at 0.032 mg/ml, confirming its strong anti- inflammatory potential. Comparator exhibited comparable IL-8 inhibitory activity (Table 6), achieving 97.17% inhibition at 0.5 mg/ml and 94.68% at 0.25 mg/ml. The inhibition was lower at 0.03 mg/ml (45.76%), yet still significant. The IC_{50} was higher than Selsun S, measured at 0.045 mg/ml, indicating potent, though less effective, IL-8 suppression.

IL-6 inhibitory activity of Selsun S and comparator

Selsun S efficiently suppressed IL-6 production in LPS-induced THP-1 cells (Table 7). At 0.5 mg/ml, inhibition reached 98.29% (O.D.=2.959±0.03). Even at 0.12 mg/ml, inhibition remained strong at 78.59% and dropped to 44.49% at the lowest tested concentration (0.03 mg/ml). The IC_{50} was calculated to be 0.033 mg/ml, reflecting its robust anti-inflammatory profile. Interestingly, Comparator displayed a comparable but reduced IL-6 inhibition profile (Table 8) relative to Selsun S across all tested concentrations. The inhibition peaked at 94.77% at 0.5 mg/ml and was 44.31% at 0.03 mg/ml. The IC_{50} was calculated to be 0.041 mg/ml, indicating lower potency than Selsun S (IC_{50}=0.033 mg/ml) (Table 9).

Table 1: Cytotoxicity analysis of Selsun S (SRC/AB/24/01/01).

Concentration (mg/ml)	O.D				SD	Viability (%)	IC_{50}
	Replicate 1	Replicate 2	Replicate 3	Average			
0.62	0.070	0.069	0.071	0.070	0.001	26.52	0.14 mg/ml
0.31	0.080	0.085	0.092	0.086	0.006	32.45	
0.15	0.096	0.083	0.110	0.096	0.010	36.45	
0.07	0.145	0.147	0.121	0.138	0.010	52.10	
0.035	0.147	0.165	0.157	0.156	0.009	59.22	
0.015	0.198	0.221	0.151	0.190	0.030	72.01	
Control	0.260	0.264	0.268	0.264	0.004	NA	

Table 2: Cytotoxicity analysis of comparator (SRC/AB/24/01/02).

Concentration (mg/ml)	O.D				SD	Viability (%)	IC_{50}
	Replicate 1	Replicate 2	Replicate 3	Average			
0.62	0.057	0.066	0.075	0.066	0.009	25.00	0.12 mg/ml
0.31	0.080	0.085	0.070	0.078	0.020	29.67	
0.15	0.115	0.124	0.125	0.121	0.020	45.96	
0.07	0.154	0.142	0.156	0.151	0.010	57.07	
0.035	0.178	0.189	0.181	0.183	0.060	69.19	
0.015	0.210	0.216	0.212	0.213	0.040	80.56	
Control	0.260	0.264	0.268	0.264	0.004	NA	

Table 3: TNF- alpha inhibitory activity of Selsun S (SRC/AB/24/01/01).

Concentration (mg/ml)	O.D			SD	Inhibition (%)	IC_{50}
	Replicate 1	Replicate 2	Average			
0.5	1.402	1.394	1.398	0.01	93.67	At concentrations <0.03 mg/ml
0.25	1.264	1.297	1.281	0.02	85.80	
0.12	1.160	1.162	1.161	0.00	77.79	
0.06	1.040	1.058	1.049	0.01	70.28	
0.03	0.994	1.014	1.004	0.01	67.27	
Control	1.526	1.459	1.493	0.04	NA	

Table 4: TNF- alpha Inhibitory activity of comparator (SRC/AB/24/01/02).

Concentration (mg/ml)	O.D			SD	Inhibition (%)	IC_{50}
	Replicate 1	Replicate 2	Average			
0.5	1.297	1.314	1.306	0.01	87.47	At concentrations <0.03 mg/ml
0.25	1.260	1.211	1.236	0.03	82.78	
0.12	1.243	1.202	1.223	0.02	81.91	
0.06	1.144	1.226	1.185	0.05	79.40	
0.03	1.081	1.167	1.124	0.06	75.31	
Control	1.526	1.459	1.493	0.04	NA	

Table 5: IL-8 inhibitory activity of Selsun S (SRC/AB/24/01/01).

Concentration (mg/ml)	O.D			SD	Inhibition (%)	IC_{50}
	Replicate 1	Replicate 2	Average			
0.5	4.472	4.568	4.520	0.06	99.82	0.032 mg/ml
0.25	4.504	4.166	4.335	0.23	95.74	
0.12	3.564	3.578	3.571	0.01	78.86	
0.06	3.064	2.812	2.938	0.17	64.89	
0.03	1.662	1.688	1.675	0.01	36.99	
Control	4.624	4.432	4.528	0.13	NA	

Table 6: IL-8 inhibitory activity of comparator (SRC/AB/24/01/02).

Concentration (mg/ml)	O.D			SD	Inhibition (%)	IC_{50}
	Replicate 1	Replicate 2	Average			
0.5	4.500	4.300	4.400	0.14	97.17	0.045 mg/ml
0.25	4.244	4.330	4.287	0.06	94.68	
0.12	3.958	3.798	3.878	0.11	85.64	
0.06	2.436	2.290	2.363	0.10	52.19	
0.03	2.138	2.006	2.072	0.09	45.76	
Control	4.624	4.432	4.528	0.13	NA	

Table 7: IL-6 inhibitory activity of Selsun S (SRC/AB/24/01/01).

Concentration (mg/ml)	O.D			SD	Inhibition (%)	IC_{50}
	Replicate 1	Replicate 2	Average			
0.5	2.937	2.981	2.959	0.03	98.29	0.033 mg/ml
0.25	2.884	2.732	2.808	0.10	93.27	
0.12	2.362	2.370	2.366	0.01	78.59	
0.06	2.052	1.935	1.994	0.08	66.22	
0.03	1.328	1.351	1.340	0.02	44.49	
Control	3.075	2.946	3.011	0.09	NA	

Table 8: IL-6 inhibitory activity of comparator (SRC/AB/24/01/02).

Concentration (mg/ml)	O.D			SD	Inhibition (%)	IC_{50}
	Replicate 1	Replicate 2	Average			
0.5	2.899	2.807	2.853	0.07	94.77	0.041 mg/ml
0.25	2.752	2.771	2.762	0.01	91.73	
0.12	2.601	2.500	2.551	0.01	84.72	
0.06	1.790	1.758	1.774	0.02	58.93	
0.03	1.081	1.587	1.334	0.35	44.31	
Control	3.075	2.946	3.011	0.09	NA	

Table 9: Summary of key IC_{50} values.

Parameter	Selsun S (mg/ml)	Comparator (mg/ml)
Cytotoxicity (MTT)	0.14	0.12
TNF-α inhibition	<0.03	<0.03
IL-8 inhibition	0.032	0.045
IL-6 inhibition	0.033	0.041

DISCUSSION

The current investigation evaluated and compared the cytotoxic and anti-inflammatory properties of two topical formulations, Selsun S and comparator, using THP-1 cells as an in vitro model (Table 9). The results reveal important insights into their safety and anti-inflammatory profiles, particularly in terms of their ability to inhibit key pro inflammatory cytokines TNF-α, IL-8, and IL-6 which play central roles in inflammation associated dermatological conditions.[18]

Both formulations exhibited dose-dependent cytotoxicity, as demonstrated by the MTT assay. Selsun S had an IC_{50} of 0.14 mg/ml, whereas comparator showed a lower IC_{50} of 0.12 mg/ml, indicating higher cytotoxic potential.[15] However, at lower concentrations (≤0.03 mg/ml), cell viability remained relatively high for both products (>70%), suggesting that therapeutically relevant concentrations may avoid cytotoxic effects. This is particularly important in topical applications where systemic absorption is minimal but local tolerability is critical.[7]

Both formulations demonstrated potent TNF-α inhibition, with IC_{50} values below 0.03 mg/ml. TNF-α is a key mediator in acute inflammation and immune regulation, making it a critical target in anti- inflammatory therapies. Notably, Selsun S showed higher maximal inhibition (93.67%) compared to comparator (87.47%) at 0.5 mg/ml. Despite this, both products retained significant activity even at the lowest tested concentration, highlighting their strong and concentration-independent TNF- suppression. This suggests potential clinical benefits in inflammatory skin disorders such as seborrheic dermatitis or psoriasis.[19]

IL-8, a chemokine responsible for neutrophil recruitment and activation, was strongly inhibited by both products. Selsun S demonstrated better IL-8 suppression, with an IC50 of 0.032 mg/ml compared to 0.045 mg/ml for comparator. Moreover, Selsun S achieved near-complete inhibition (99.82%) at 0.5 mg/ml. While both products showed activity, the more rapid decline in comparator's inhibition at lower concentrations suggests a potentially narrower effective dose range for IL-8 modulation. This could influence its effectiveness in treating conditions with dominant neutrophilic inflammation.[20]

Selsun S and comparator both exhibited strong IL-6 inhibition profiles; however, Selsun S demonstrated higher potency, with an IC_{50} of 0.033 mg/ml compared to 0.041 mg/ml for comparator. While both formulations achieved over 90% inhibition at higher concentrations, the lower IC_{50} of Selsun S suggests more effective suppression of chronic inflammation mediators at lower doses. This distinction, though modest, may be relevant in optimizing topical therapeutic concentrations.[21]

Both formulations exhibited comparable suppression of TNF-α, a key pro-inflammatory cytokine. While both were effective in reducing IL-6 levels, Selsun S showed greater potency, as evidenced by its lower IC_{50} value. Selsun S also outperformed comparator in suppressing IL-8, which plays a crucial role in chronic inflammation and immune cell recruitment. The presence of 3% salicylic acid in Selsun S may contribute to enhanced penetration and anti-inflammatory activity, while selenium sulfide is known to modulate immune responses in addition to its antifungal action. This suggests that Selsun S may offer dual benefits—antimicrobial and anti-inflammatory—making it a favorable option in treating inflammatory dermatoses.

Both Selsun S and comparator are effective in vitro anti-inflammatory agents; however, Selsun S demonstrated superior activity, particularly in inhibiting IL-8 and more effectively inhibiting IL-6. These results support the potential clinical utility of selenium disulfide-based formulations for managing inflammatory skin conditions.[21]

CONCLUSION

This *in vitro* study demonstrates that both Selsun S (1% selenium disulfide + 3% salicylic acid) and the comparator formulation (2% ketoconazole + 2% salicylic acid) effectively suppress key pro-inflammatory cytokines involved in scalp inflammation. Selsun S exhibited

superior inhibition of IL-6 and IL-8, along with lower cytotoxicity, suggesting enhanced anti-inflammatory efficacy and better tolerability. These effects are likely attributable to the synergistic action of selenium disulfide and a higher concentration of salicylic acid, which together improve skin penetration and offer antimicrobial benefits. Collectively, these findings support the potential clinical utility of selenium disulfide-based formulations in the management of inflammatory dermatological conditions.

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