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Original Article

In vitro Effects of Acetaldehyde and Cotinine on Proliferation and Secretory Activity of Rat Umbilical Cord-Derived Mesenchymal Stem Cells

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Abstract

Introduction: Umbilical cord–derived mesenchymal stem cells (UCMSCs) are widely utilized in regenerative medicine owing to their proliferative and secretory capabilities. However, the effects of alcohol- and tobacco-derived metabolites, specifically acetaldehyde and cotinine, on UCMSC viability and function remain poorly understood. This study aimed to compare the cytotoxic and secretory responses of rat UCMSCs following *in vitro* exposure to acetaldehyde and cotinine.

Materials and Methods: Rat UCMSCs were cultured in DMEM/F12 and seeded at 5×10^5 cells per T-25 flask. A total of 40 flasks were allocated to three groups: acetaldehyde (30 µM, n = 15), cotinine (14 µM, n = 15), and untreated controls (n = 10). Cells were incubated for 5 days under standard conditions. Cell counts were recorded daily, and the levels of VEGF, HGF, IGF-1, SDF-1/CXCL12, IL-6, TNF-α, and IL-1β were quantified using a multiplex immunoassay. Statistical analysis was performed using two-way ANOVA.

Results: Acetaldehyde exposure resulted in a progressive decline in UCMSC numbers from 4.8×10^5 to 2.8×10^5 over 5 days, whereas cotinine increased cell numbers from 5.4×10^5 to 9.0×10^5. Acetaldehyde induced an early peak, followed by subsequent declines in VEGF, HGF, IGF-1, SDF-1/CXCL12, and IL-6, while TNF-α and IL-1β showed variable patterns. In contrast, cotinine led to significantly less expansion compared with untreated controls, although it did induce a modest increase from baseline.

Conclusions: Acetaldehyde exerts a markedly more pronounced cytotoxic and suppressive effect on rat UCMSCs compared with cotinine, which induces a gradual increase in cell proliferation and secretory activity. However, cotinine's effect is less inhibitory than acetaldehyde, yet it still significantly reduces expansion relative to untreated cells. These findings underscore the differential biological impacts of alcohol- and tobacco-related metabolites on stem cell function *in vitro*.

Keywords: Mesenchymal Stem Cells, Umbilical Cord, Acetaldehyde, Cotinine, Cell Proliferation, Cytokines

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Introduction

Tobacco smoking and alcohol consumption are among the most prevalent lifestyle-related risk factors worldwide and have each been independently associated with systemic toxicity. The pathophysiological consequences of these exposures extend beyond localized pulmonary and hepatic injury to encompass global metabolic dysregulation. Among the most notable secondary metabolites generated are cotinine and acetaldehyde.[1]

Following systemic accumulation after exposure, both compounds are thought to contribute to inflammatory dysfunction. Cotinine, in particular, exhibits a prolonged half-life of 15–20 hours, facilitating systemic persistence even long after smoking cessation.[3-5] Conversely, acetaldehyde is a direct hepatic metabolite of ethanol and is known to modify proteins and nucleic acids.[6] Previous reports indicate that cotinine inhibits UCMSC proliferation and paracrine factor secretion,[5] while both cotinine and acetaldehyde can compromise pluripotent progenitor activity.[2]

Umbilical cord–derived mesenchymal stem cells (UCMSCs) are characterized by substantial proliferative capacity, low immunogenicity, and robust paracrine secretion of anti-inflammatory cytokines.[7,8] Understanding the molecular mechanisms through which cotinine and acetaldehyde exert their effects is essential for elucidating their roles in oxidative damage and inflammatory responses.[9,10]

Despite extensive toxicological studies on differentiated somatic cells, comparative data on the effects of these two metabolites on UCMSCs remain scarce. Previous investigations have typically assessed each metabolite in isolation, and the specific impact of acetaldehyde and cotinine on UCMSC viability and secretory function is still poorly understood. Given the therapeutic relevance of UCMSCs, addressing this

knowledge gap is of considerable scientific importance.

Accordingly, this study aimed to comparatively evaluate the cytotoxic effects of acetaldehyde and cotinine on rat UCMSCs *in vitro*, using cell counts and the levels of selected proteins, VEGF, HGF, IGF-1, SDF-1/CXCL12, IL-6, TNF-α, and IL-1β, as functional parameters.

Materials and Methods

Cell Sourcing, Preparation, and Culture

Rat umbilical cord–derived mesenchymal stem cells (UCMSCs) were obtained commercially (Catalogue No. abx700046, Abbexa®, UK). The identity of the cells was confirmed by flow cytometry, showing positivity for CD29, CD44, CD90, CD73, and CD105, and negativity for CD34 and CD45. Upon receipt, cell viability was >85%, and the cells tested negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi. The cryopreserved cells were rapidly thawed in a 37 °C water bath with gentle agitation. The cell suspension was then centrifuged at 1000 RPM for 5 minutes, the supernatant was discarded, and the cell pellet was washed once with phosphate-buffered saline (PBS). The culture medium used for routine maintenance consisted of DMEM/F12 supplemented with 5% fetal bovine serum (FBS), 1% growth supplement, and 1% penicillin–streptomycin, and cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. For subculturing, adherent cells were detached using 1 mL of trypsin at 37 °C, followed by neutralization with fresh culture medium and passaging at a 1:2 ratio. Cell counts were determined using an automated cell counter (Countess™ II FL; Invitrogen). For the experiments, cells were seeded at a density of 5×10^5 cells per T-25 flask. A total of 40 independent T-25 flasks were allocated to the following groups: fifteen flasks treated with acetaldehyde (30 µM; Sigma-Aldrich; ≥ 99.5% purity), fifteen flasks treated with cotinine (14 µM; Sigma-Aldrich; ≥ 98% purity), and ten flasks serving as untreated controls (0.1% DMSO in DMEM/F12).[11]

Culture Conditions

All cultures were maintained in a humidified CO_2 incubator (HERAcell VIOS 160i; Thermo Fisher Scientific) programmed to 37 °C ± 0.5 °C, 5% CO_2 ± 0.1%, and 95% relative humidity. The pH was maintained between 7.35 and 7.45 using a pH electrode (Accumet; Fisher Scientific), and cultures were kept under hypoxic conditions (~5% O_2). The culture medium was exchanged every 24 hours by adding 5 ml of fresh supplemented DMEM/F12. The increase in cell count observed in the control group was attributed to the hypoxic environment (5% O_2), which enhances proliferation and reduces doubling time.[12] Biological replicates (n = 40) were independently established and monitored throughout the experimental period. Repeated measurements at different time points were obtained from the same cultures. Following seeding, UCMSCs were incubated for 24 h to allow cell attachment. Thereafter, the culture medium was replaced with fresh medium containing acetaldehyde or cotinine.

Cell Standardization and Treatment

All experimental flasks were standardized to contain the same cell density at the time of acetaldehyde and cotinine exposure. Cultures in the treatment groups received acetaldehyde (30 µM) or cotinine (14 µM), respectively. The selection of these concentrations was based on biologically relevant exposure ranges reported in the literature, together with preliminary experimental optimization, to induce measurable cellular responses while avoiding excessive cytotoxicity.[13] Previous studies have demonstrated that cigarette smoke constituents and nicotine-related metabolites impair mesenchymal stem cell proliferation, differentiation, oxidative balance, and regenerative capacity. Accordingly, the chosen concentrations represented subcytotoxic levels intended to elicit detectable alterations in MSC biological behavior without causing immediate or complete cell death. The present study was designed as a model of short-term, acute metabolic exposure; therefore, higher concentrations were deliberately avoided to prevent nonspecific cytotoxicity and significant cell loss. Following standardization, cells were seeded at 5×10^5 cells per T-25 flask and maintained for 5 days. On day 0, adherent UCMSCs from passage-5 cultures were dissociated using 0.25% trypsin-EDTA for 5 minutes at 37 °C in FBS-supplemented DMEM/F12, followed by centrifugation at 300 × g for 5 minutes. Cell counts were determined on each study day using an automated cell counter (Countess™ II FL; Invitrogen) with a fluorescent viability stain.

Cell Harvesting and Quantification

Culture supernatants were aspirated from the flasks on each study day and centrifuged at 1,200 × g for 10 minutes at 4 °C. The resulting supernatants were stored as a batch at −80 °C until analysis. Adherent cells were washed three times with phosphate-buffered saline (PBS) and then incubated with trypsin-EDTA at 37 °C. Viable cell counts were normalized to the initial seeding density to calculate cell expansion.[14]

Multiplex Immunoassay for Protein Quantification

All assays were performed according to the manufacturer's instructions, and analytical performance characteristics were within acceptable ranges for bioanalytical validation. Levels of VEGF, HGF, IGF-1, SDF-1/CXCL12, IL-6, TNF-α, and IL-1β were quantified in clarified culture supernatants using a high-sensitivity multiplex sandwich immunoassay (Luminex® xMAP; MilliporeSigma). Clarified culture supernatants were thawed on ice and centrifuged at 10,000 × g for 10 minutes at 4 °C prior to the assay. Each sample was

incubated with 25 µl of antibody-conjugated bead suspension at room temperature for 60 minutes with shaking. The wells were then washed three times with 150 µl of wash buffer (0.1 M PBS, pH 7.4, containing 0.05% Tween-20) using a vacuum manifold (MultiScreen; Millipore). Detection antibodies (biotinylated, species-specific monoclonal antibodies; 25 µl per well at optimized concentrations) were subsequently incubated for 30 minutes. After washing, 50 µl of streptavidin–phycoerythrin conjugate was added to each well and incubated for 15 minutes at room temperature. Analyte concentrations were determined using a Luminex® 200 analyzer (Luminex Corporation, Austin, TX, USA), with beads classified by color (red laser excitation at 635 nm). Median fluorescence intensities (MFI) were plotted against diluted recombinant standards. Cell counts were performed daily, and secretion rates were expressed as pg/ml. Each assay was performed in triplicate. All analytes were measured within the assay's linear dynamic range to ensure quantifiable detection above the lower limit of detection (LLOD).[15]

Statistical Analysis

All numerical data were processed using SPSS (version 27) and Microsoft Excel 2019, and mean values were computed for each treatment group and time point. Kinetic curves for cell number and protein secretion were generated as mean values across biological replicates (n = 40), with line graphs plotted with 95% confidence intervals. Comparisons of peak protein levels and time-to-peak between the acetaldehyde and cotinine groups were performed using two-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) post hoc test for pairwise contrasts. Statistical significance was set at $p \leq 0.05$.

Results

Over the five-day observation period, the acetaldehyde-treated group demonstrated a progressive decline in cell counts from 4.8×10^5 on day 1 to 2.8×10^5 on day 5, representing a 41.7% decrease. In contrast, the cotinine-treated group showed less inhibition than acetaldehyde; however, cell proliferation remained markedly reduced compared with normal UCMSC proliferation, increasing from 5.4×10^5 to 9.0×10^5 (a 66.7% increase) relative to the control group (Table 1, Figure 1).

Table 1. Cell Counts of Rat UCMSCs Following Exposure to Acetaldehyde, Cotinine, or Control Over the 5-Day Observation Period

Test Substance	Cell count ($\times 10^5$)					
	Baseline day	1 day	2 days	3 days	4 days	5 days
Acetaldehyde (30) µM	5aA	4.8aA	4.2bA	3.6cA	3dA	2.8dA
cotinine (14) µM	5aA	5.4bB	6.3cB	7dB	8.1eB	9fB
Control	5aA	6bC	9.6cC	17dC	24eC	31fC
LSD ($p < 0.05$)	**0.212×10^5**					

Uppercase letters indicate significant differences between columns, and lowercase letters indicate significant differences between rows. Values sharing the same letter (either uppercase or lowercase) are not significantly different, whereas values with different letters differ significantly ($p \leq 0.05$).

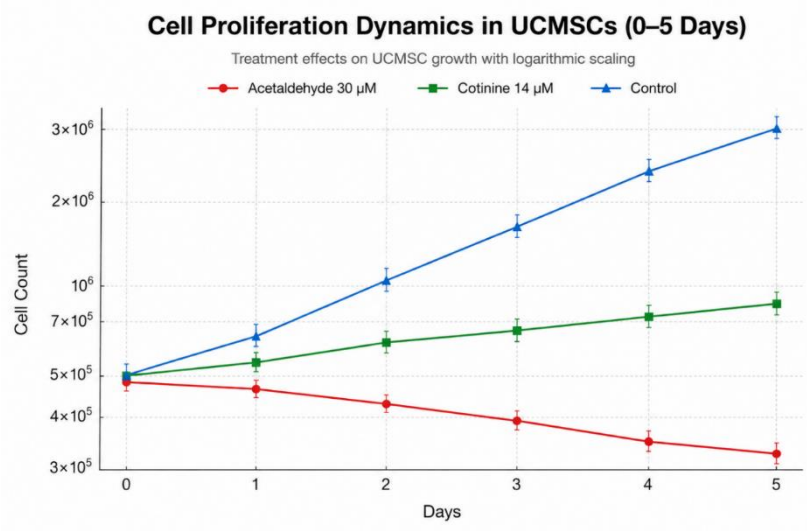

Figure 1. Cell Proliferation Curves for UCMSCs Exposed to Acetaldehyde (red line), Cotinine (green line), and Untreated Control (blue line) Over 5 Days. Cell counts are shown on a logarithmic scale.

Exposure of UCMSCs to acetaldehyde resulted in dysregulation of secreted proteins over the 5-day culture period (pg/10^5 cells). VEGF exhibited an early increase from 378 pg/ml on day 1 to a peak of 840 pg/ml by day 3, followed by a decline to 533 pg/ml by day 5, representing a maximum increase of 122%. HGF rose from 520 pg/ml on day 1 to 1,489 pg/ml on day 4 (a 187% increase), followed by a partial regression to 1,345 pg/ml on day 5. IGF-1

peaked at 177 pg/ml on day 3 before declining to 110 pg/ml on day 5, falling below the day-1 value of 125 pg/ml. SDF-1/CXCL12 peaked at 417 pg/ml on day 3 (a 132% increase over the day-1 baseline), with a subsequent sharp decline to 230 pg/ml on day 5. IL-6 increased from 620 pg/ml on day 1 to a peak of 1,983 pg/ml on day 3 (a 220% increase), followed by partial resolution to 1,100 pg/ml on day 5. In contrast, TNF-α remained relatively stable throughout the observation period, ranging between 30 and 54 pg/ml, and returned to baseline by day 5. IL-1β declined progressively from 20 pg/ml on day 1 to 11 pg/ml on day 5 (Table 2).

Table 2. Secreted Protein Levels (VEGF, HGF, IGF-1, SDF-1/CXCL12, IL-6, TNF-α, and IL-1β) in rat UCMSCs Over the 5-Day Exposure Period to Acetaldehyde

Toxic / Proteins	Acetaldehyde (30 pg/ml)							LSD (pg/ml)
	Control	Baseline day	1 day	2 days	3 days	4 days	5 days	
VEGF	378a	378a	489b	637c	840d	730e	533f	3.68
HGF	520a	520a	684b	936c	1307d	1489e	1345f	2.88
IGF-1	95a	95a	125b	146c	177d	142e	110f	2.14
SDF-1 / CXCL12	180a	180a	228b	328c	417d	339e	230f	3.65
IL-6	620a	620a	937b	1477c	1983d	1438e	1100f	5.06
TNF-α	30a	30a	41b	54c	43d	35e	30a	1.23
IL-1β	20a	20a	20a	29b	24c	17d	11e	0.98

Different letters within the same row indicate statistically significant differences, whereas identical letters indicate non-significant differences.

Exposure to cotinine (14 µM) elicited a distinct secretory kinetic profile, characterized by a steady, linear elevation across all proteins over the 5-day period, with no terminal decline observed (pg/10^5 cells). VEGF rose from 378 pg/ml to 566 pg/ml by day 5, representing a 50% increase. HGF showed modest but consistent upregulation from 520 to 724 pg/ml over 5 days (a 39% increase). IGF-1 also exhibited a sustained elevation from 95 to 159 pg/ml (a 67% increase). SDF-1/CXCL12 escalated monotonically from 180 pg/ml to a peak of 666 pg/ml by day 5, representing a 270% increase. IL-6 rose from 620 to 900 pg/ml (a 45% increase). TNF-α and IL-1β showed parallel progressive increases, reaching 58 and 41 pg/ml by day 5, respectively (93% and 105% increases), as summarized in Table 3.

Table 3. Secreted Protein Levels (VEGF, HGF, IGF-1, SDF-1/CXCL12, IL-6, TNF-α, and IL-1β) in Rat UCMSCs Over the 5-Day Exposure Period to Cotinine

Test substance	Cotinine (14 pg/ml)							LSD
	Control	0 day	1 day	2 days	3 days	4 days	5 days	
VEGF	378a	378a	395b	419c	488d	510e	566f	3.02
HGF	520a	520a	559b	590c	644d	699e	724f	2.66
IGF-1	95a	95a	110b	124c	136d	148e	159f	1.74
SDF-1/CXCL12	180a	180a	260b	312c	388d	480e	666f	4.12
IL-6	620a	620a	640b	676c	705d	810e	900f	5.18
TNF-α	30a	30a	35b	41c	45d	51e	58f	1.21
IL-1β	20a	20a	24b	31c	33d	37e	41f	1.36

For comparisons within the same row, values with different lowercase superscripts differ significantly ($p \leq 0.05$), while those sharing the same lowercase superscript do not differ significantly.

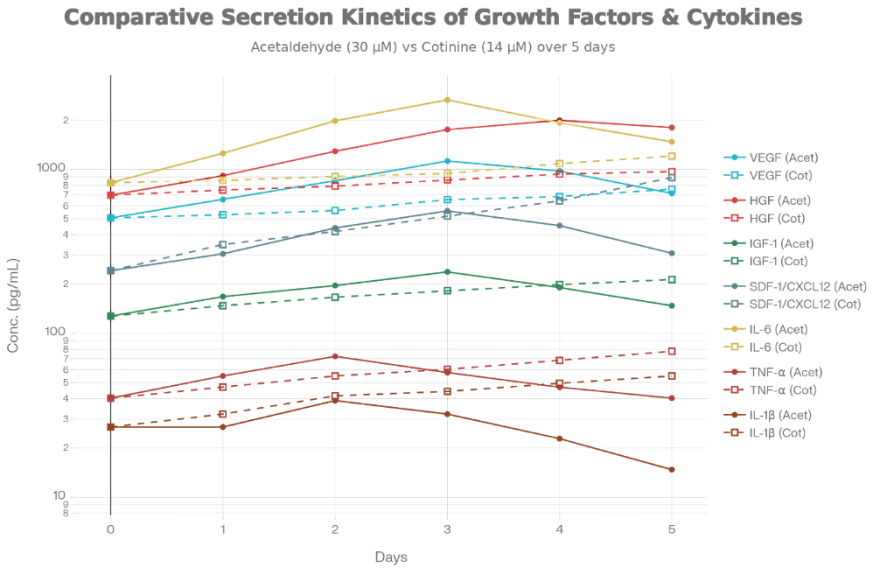

Figure 2. Comparative Profiles of Secreted Protein Levels (VEGF, HGF, IGF-1, SDF-1/CXCL12, IL-6, TNF-α, and IL-1β) in Rat UCMSCs Exposed to Acetaldehyde and Cotinine Over the 5-Day Culture Period. Values are expressed as mean ± SD. Different bar patterns distinguish the treatment groups, and the figure has been optimized for clarity in grayscale printing.

A comparison of the effects of cotinine and acetaldehyde on protein secretion in rat UCMSCs over 5 days revealed distinct kinetic profiles. Cotinine induced a gradual and sustained increase in protein levels throughout the observation period, whereas acetaldehyde elicited rapid and pronounced increases, peaking on days 3 and 4 and subsequently declining markedly by day 5 (Figure 2).

Discussion

Accurate characterization of the stem cell molecular microenvironment, particularly the secretory profile of mesenchymal stem cells, is fundamental to regenerative biology. Acetaldehyde and cotinine accumulate in body tissues and have been shown to affect paracrine signaling networks in UCMSCs.[16]

In our results, the number of UCMSCs decreased progressively in the acetaldehyde-treated group over the five-day period, whereas cotinine-treated cultures exhibited a small but consistent increase compared with untreated controls. The concentrations used in this study were selected based on biologically relevant exposure ranges reported in the literature. Circulating cotinine levels in habitual smokers typically range from 10 to 500 ng/ml, corresponding to nanomolar to low micromolar concentrations. Acetaldehyde, by contrast, transiently reaches micromolar concentrations in the blood following ethanol consumption but is rapidly metabolized. Thus, the chosen concentrations (acetaldehyde 30 μM and cotinine 14 μM) represent a controlled in vitro exposure model designed to elicit measurable cellular responses while avoiding excessive cytotoxicity. Acetaldehyde is known to inhibit MSC proliferation and markedly reduce proliferative activity.[17] The progressive decline in stem cell numbers observed following acetaldehyde exposure is consistent with previous reports.[16] Mechanistically, acetaldehyde reduces cell counts and lowers ATP levels, which are essential for cell division. Furthermore, it interacts directly with proteins and DNA, forming adducts that inhibit DNA replication,[18] and can block cell-proliferation signaling pathways.[19]

The impact of cotinine on MSC number is dependent on the specific substance, exposure concentration, and duration. In the present study, cotinine exposure did not induce a significant deviation in cell numbers relative to the control group, although the observed increase was not as pronounced as that seen in untreated cultures. Cotinine has been reported to inhibit proliferation compared with untreated controls;[20] conversely, prolonged exposure or higher concentrations may slightly retard the increase in cell number due to cumulative effects on cell cycle regulation.[21] Although a reduction in total cell number was observed, this parameter alone does not allow precise discrimination between decreased proliferation and cell cycle arrest. Therefore, the absence of complementary viability assays, such as MTT/CCK-8 or apoptosis detection assays (Annexin V/PI), limits the mechanistic interpretation of the observed effects.

Based on our results, cotinine induced a steady, linear elevation in VEGF, HGF, IGF-1, SDF-1/CXCL12, IL-6, TNF-α, and IL-1β in rat UCMSCs over 5 days, with no terminal decline observed. Interestingly, cotinine resulted in a 66% increase in cell numbers from baseline. Although cotinine is considered to have relatively weak biological toxicity, this finding suggests that at low concentrations it may exert non-cytotoxic and even proliferative effects—a phenomenon consistent with a hormetic dose response, wherein low-level exposure induces adaptive cellular responses. Cotinine is known to modulate cell survival pathways, including PI3K/Akt and MAPK signaling, which may contribute to enhanced MSC proliferation under specific in vitro conditions. Therefore, the observed increase in cell count likely reflects a dose-dependent adaptive response rather than a direct proliferative stimulus or a purely cytotoxic effect.

The effect of cotinine on MSC proliferation depends on the concentration and duration of exposure. At low concentrations, cotinine induces a slight increase in the division rate,[2] an effect associated with mild activation of cell survival pathways.[9] Conversely, higher concentrations result in suppressed cell proliferation.[22,23] All experimental groups were maintained under hypoxic conditions (5% O_2); therefore, the observed differences in proliferation cannot be attributed to variations in oxygen tension. Hypoxia enhances MSC proliferation by stabilizing HIF-1α and activating glycolysis. However, acetaldehyde exposure may override hypoxia-induced proliferative signaling through excessive reactive oxygen species (ROS) production. In contrast, cotinine appears to exert a less cytotoxic, and potentially adaptive, hormetic effect, which may allow hypoxia-driven proliferative signaling to persist. These findings suggest differential interactions between acetaldehyde, cotinine, and hypoxia-regulated signaling pathways. Regarding the acetaldehyde-treated group, VEGF, HGF, IGF-1, SDF-1/CXCL12, and IL-6 levels were elevated during the first three days, followed by a decline over the subsequent two days. TNF-α remained relatively stable, with modest fluctuations before returning to baseline by day 5. IL-1β increased on days 1 and 2, followed by a decline by the end of day 3.

Under continuous exposure conditions, acetaldehyde stimulated the secretion of IL-6, TNF-α, and IL-1β, albeit with distinct temporal patterns. Specifically, IL-6 levels increased over the initial days of exposure, whereas TNF-α declined over longer periods.[24] Furthermore, acetaldehyde induced a gradual down-regulation of growth-related gene expression while simultaneously elevating the expression of other functional genes.[25,26] In the cotinine-treated group, VEGF levels increased progressively over the 5-day period.

Similarly, modest yet consistent increases were observed for HGF, IGF-1, SDF-1/CXCL12, and IL-6, whereas TNF-α and IL-1β showed progressive elevation. The elevated VEGF, HGF, and IGF-1 levels following cotinine exposure suggest indirect activation of cell survival pathways without overstimulation of secretory activity. SDF-1/CXCL12, which is involved in essential processes such as cell migration and microenvironmental interactions, was maintained within the physiological range under cotinine exposure.[27] In contrast, the responses of IL-6, TNF-α, and IL-1β to cotinine were more variable. Cotinine generally exhibited anti-inflammatory properties, slightly reducing the levels of these cytokines; nevertheless, prolonged exposure could elicit a limited inflammatory response.[28]

IL-1β in the acetaldehyde-treated group exhibited temporal fluctuations over the 5-day observation period, suggesting a dynamic inflammatory response. The initial elevation likely reflects acute cellular stress, which is subsequently followed by adaptive cellular responses. Overall, acetaldehyde appears to induce progressive inflammatory dysregulation in UCMSCs.[29] In the acetaldehyde-treated group, secretory responses were rapid and pronounced, peaking on day 3 and subsequently declining on days 4 and 5. In contrast, cotinine exposure elicited a progressive, time-dependent, and sustained rise in most proteins, followed by gradual stabilization. While changes in VEGF and HGF levels may be partially attributable to alterations in total cell number, it is also plausible that the tested compounds exert direct regulatory effects on cellular secretory pathways, including modulation of gene expression and paracrine signaling machinery. Accordingly, the observed shifts in protein levels likely reflect a functional reprogramming of cellular secretory activity.

UCMSCs were markedly susceptible to acetaldehyde, which led to significant reductions in VEGF, HGF, and IGF-1, whereas cotinine maintained these proteins at levels comparable to those in the control group. Conversely, acetaldehyde elevated IL-6, TNF-α, and IL-1β relative to the control, and cotinine increased these cytokines to a lesser extent, suggesting that acetaldehyde exerts a more pronounced pro-inflammatory effect than cotinine.[30] The peak in protein secretion observed on day 3 in the acetaldehyde-treated group, followed by a subsequent decline, may reflect a dynamic cellular response to sustained toxic stress. Alternative explanations include the induction of progressive cellular senescence or functional exhaustion following prolonged acetaldehyde exposure, or an initial compensatory upregulation in response to acute stress. Distinguishing among these possibilities would require additional assays to assess senescence markers, metabolic activity, and changes in culture medium composition over time.

The observed alterations in cytokine levels suggest that acetaldehyde and cotinine may differentially influence signaling pathways associated with UCMSC functions. However, these findings should not be interpreted as direct evidence of enhanced or impaired regenerative capacity in the absence of functional assays.

Although previous studies have investigated the individual effects of acetaldehyde or cotinine on MSCs, the present study provides a controlled in vitro model rather than a direct replication of physiological exposure. Circulating cotinine levels in habitual smokers typically range from approximately 10 to 500 ng/ml, corresponding to nanomolar to low micromolar concentrations, whereas acetaldehyde concentrations in blood transiently reach micromolar levels following moderate alcohol consumption but are rapidly metabolized. Therefore, the exposure conditions used herein should be interpreted as experimental rather than strictly physiological.

A limitation of this study is the reliance on total cell count as the sole endpoint for evaluating cellular responses. Additional functional assays assessing cell viability and apoptosis would provide a more comprehensive understanding of the underlying mechanisms.

Conclusion

Acetaldehyde exerted a more deleterious effect on UCMSCs than cotinine, indicating a stronger suppressive influence on cell viability and secretory function, with potential adverse effects on cellular function. Cotinine exhibited a comparatively weaker inhibitory effect. However, the clinical translatability of these findings is limited, as the study was conducted in vitro and did not include functional regenerative assays or in vivo validation. Future studies should investigate the effects of acetaldehyde and cotinine on UCMSC functional properties, including migration, differentiation, and angiogenic capacity, and explore the underlying molecular mechanisms responsible for the observed differential cellular responses.

Authors' Contributions

GKE: Conceptualization, methodology, investigation, data curation, writing – original draft, and supervision; NMM: Writing – review & editing and formal analysis. All authors have read and approved the final manuscript.

Ethical Approval

All experimental procedures were conducted in accordance with institutional guidelines and approved by the Ethical Committee of the College of Veterinary Medicine, University of Al-Qadisiyah, under approval number 622, issued on 10 February 2026. The study adhered to internationally accepted standards for the care and use of laboratory animals.

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Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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