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Original research

E-liquids vs. cigarettes: mexican analysis

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ABSTRACT

Introduction: The combustion of tobacco produces thousands of toxic compounds, many of which are recognized carcinogens. In contrast, electronic cigarettes (ECs) heat e-liquids at lower temperatures without combustion, potentially reducing exposure. **Objetive**: This study aimed to assess the presence of five compounds of concern diacetyl, formaldehyde, acetaldehyde, benzaldehyde, and vitamin E acetate (VEA) in commercial e-liquids and to qualitatively compare their chemical profile with combustible cigarette smoke. **Methods:** Twenty e-liquids and one combustible cigarette brand were analyzed. E-liquids and Cigarette smoke were examined using Headspace Gas Chromatography with Flame Ionization Detection and Gas Chromatography–Mass Spectrometry (GC-MS). Identification was based on retention times and spectra compared with pure standards. Compounds were classified according to the Hazardous Substances Data Bank (HSDB) and the Globally Harmonized System (GHS). **Results:** None of the five target compounds were detected in e-liquids. A total of 24 compounds were identified, with 1.7% classified as carcinogenic and 5.5% as toxic. In contrast, cigarette smoke contained 27 compounds, with 10.2% carcinogenic and 12.7% toxic, dominated by aldehydes, ketones, and polycyclic aromatic hydrocarbons (PAHs) linked to tobacco pyrolysis. The lower operating temperatures of ECs (≤ 250 °C) and the absence of combustion likely explain the reduced toxic burden observed. **Conclusion:** Commercial e-liquids presented a less hazardous chemical profile compared to combustible cigarette smoke, supporting their potential as lower-risk alternatives. However, EC aerosols are not free of health risks. Further quantitative studies, simulations of realistic use, and long-term toxicological evaluations are warranted to assess residual risks and confirm their contribution to harm reduction strategies.

Key words: electronic cigarettes; e-liquids; gas chromatography; toxic compounds; harm reduction; diacetyl; formaldehyde.

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RESUMEN

Introducción: La combustión del tabaco produce miles de compuestos tóxicos, muchos de los cuales son carcinógenos reconocidos. En contraste, los cigarrillos electrónicos (ECs) funcionan calentando e-líquidos a temperaturas sustancialmente más bajas, lo que puede reducir la exposición de los usuarios a sustancias nocivas. **Objetivo:** Nuestro objetivo es evaluar la presencia de diacetilo, formaldehído, acetaldehído, benzaldehído y acetato de vitamina E en e-líquidos comerciales comparando su perfil químico con el del humo de cigarrillos combustibles. **Métodos:** Se analizaron veinte e-líquidos y una marca de cigarrillos combustibles. Los e-líquidos y el humo de cigarrillos fueron examinados cualitativamente utilizando cromatografía de gases por ionización de llama y espectrometría de masas. La identificación se basó en los tiempos de retención y los espectros comparados con estándares puros. Los compuestos fueron clasificados de acuerdo con el "*Hazardous Substances Data Bank*" y al "*Globally Harmonized System*". **Resultados:** Los compuestos objetivo en los e-líquidos no se detectaron. Se identificaron 24 compuestos, 1.7% carcinogénicos y 5.5% tóxicos. En los cigarrillos se identificaron 27 compuestos, 10.2% carcinogénicos y 12.7% tóxicos. Las temperaturas más bajas de operación de los ECs (≤ 250 °C) y la ausencia de combustión probablemente explican la menor carga tóxica observada en los e-liquidos. **Conclusión:** Los e-líquidos comerciales presentaron un perfil químico menos peligroso lo que respalda su potencial de menor riesgo. Sin embargo, los aerosoles en los ECs no están libres de riesgo. Se requieren estudios cuantitativos adicionales y evaluaciones toxicológicas de largo plazo para valorar riesgos residuales y confirmar su papel en la reducción de daños.

Palabras clave: cigarrillos electrónicos; e-líquidos; cromatografía de gases; compuestos tóxicos; reducción de daños; diacetilo; formaldehído.

INTRODUCTION

According to the World Health Organization (WHO), cigarette smoking remains one of the leading threats to global public health, accounting for more than 8 million deaths annually. Approximately 7 million of these deaths result from direct tobacco use, while an additional 1.3 million are attributed to second-hand smoke exposure.[1] Cigarettes contain numerous harmful substances, including nicotine, tar, ammonia, and carbon monoxide.[2] Moreover, tobacco comprises over 2,550 known chemical constituents, and tobacco smoke contains more than 4,000 compounds; among them, 43 have been confirmed as carcinogens.[3] The adverse health effects of smoking have been well documented since at least 1977, with widespread awareness of its systemic risks.[4] In recent decades, the landscape of nicotine consumption has undergone a significant transformation, as the reduction or elimination of toxicants is increasingly seen as a viable strategy for disease prevention.[5] The longstanding dominance of conventional combustible cigarettes —shaped by cultural, social, and historical forces— is now being gradually displaced by emerging technologies that offer alternative inhalation-based nicotine delivery systems, most notably electronic cigarettes (ECs).[4] Also referred to as vapes or electronic nicotine delivery systems (ENDS), these devices have gained considerable popularity, largely due to discourse framing them as less harmful substitutes for traditional tobacco smoking.[4]

E-liquids used in ECs primarily consist of glycerin, propylene glycol, flavorings (which vary by product), and may or may not contain nicotine. Some formulations also may or may not contain potentially harmful carbonyls such as diacetyl, formaldehyde, acetaldehyde, and benzaldehyde -all of which are compounds of interest in this study. In recent years, heavy metals (e.g., nickel, cadmium, chromium) and organic chemicals such as pesticides, aromatic hydrocarbons, and carbonyl groups have also been detected in these products.[6] *In vitro* studies exposing human cell lines to flavored e-liquids have demonstrated oxidative stress, inflammatory responses, and disruption of pulmonary barrier function, including reduced cell viability and cell count, alterations in pro-inflammatory biomarkers, and increased cytokine release.

Diacetyl, widely used as a flavoring agent, has been linked to bronchiolitis obliterans, a severe and irreversible pulmonary condition.[2,7] Formaldehyde and acetaldehyde are known respiratory irritants and potential carcinogens. Benzaldehyde, though a common flavoring compound, has demonstrated cytotoxicity depending on dose and route of exposure. Furthermore, vitamin E acetate (VEA) has been implicated in severe lung injury when inhaled via vaping products (VPs).[8] VEA is typically found in unregulated THC-containing cartridges, where it is used as a thickening agent for THC oil.[9]

According to the National Youth Tobacco Survey from the Center for Disease Control and Prevention (CDC) the use

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of ECs among adolescents increased significantly between 2019 and 2020.[10] In response, a 2019 study analyzed 206 EC liquid samples, finding that 71% contained THC and 69% VEA.[11] This finding was central to the identification of EVALI (E-cigarette or Vaping Product Use-Associated Lung Injury) and the subsequent link to VEA-containing VPs. Symptoms of EVALI include dyspnea, cough, fever, constitutional symptoms, gastrointestinal disturbances, and hemoptysis.[9] Confirmed cases typically involved patients with a history of vaping within the previous 90 days, imaging findings (e.g., bilateral pulmonary infiltrates or ground-glass opacities on chest CT), and exclusion of infectious, neoplastic, or rheumatologic etiologies.[9] As of 2020, the CDC had reported 2,807 EVALI cases and 68 confirmed deaths, although these figures have not been updated at the time of this publication.[12]

Regardless of EVALI, expert opinions indicate that other potential health risks associated with ECs may arise from multiple sources, including the e-liquid itself, chemical reactions within the heating element, and the device's components. User behavior also influences exposure levels. The combination of these factors increases the risk of addiction, poisoning, and inhalation-related toxicity. Potential health effects may depend on specific chemical constituents or device characteristics, meaning that risks can vary across different EC products. Despite certain methodological limitations, current evidence suggests that ECs use by non-smokers especially youth is harmful, while for many major health outcomes, the overall effects of ECs remain uncertain.[13]

Methods that assess health risks based on individual component toxicity may misestimate exposure risks because interactions between components are not well studied.[14] To detect and quantify harmful compounds in ECs, several studies highlight the importance of advanced analytical techniques such as gas chromatography–mass spectrometry (GC-MS).[6] The concentration and toxicity of inhaled substances are closely related to both, the chemical formulation of the e-liquids and technical parameters involved in vaporization.[15,16] In contrast to conventional cigarettes, which combust tobacco via oxidative reactions at temperatures ranging from 600°C to 950°C, ECs heat liquids via an electrically powered coil without requiring combustion or oxygen.[17] This generates aerosols by evaporation, typically at temperatures below 250°C, thus reducing the formation of thermally degraded byproducts commonly found in cigarette smoke.[16-18]

The combustion of cigarettes produces a complex mixture of gases, free radicals, tar and solid particles through the pyrolysis of tobacco, paper and additives. In conditions of limited oxygen availability, incomplete combustion may result in the formation of hazardous substances, such as carbon monoxide (CO), polycyclic aromatic hydrocarbons (PAHs) and aldehydes.[17] Pyrolysis studies have shown that volatile compounds can be released at temperatures as low as 180°C, while PAHs and CO emerge between 300–650°C and above 600°C, respectively.[18]

Under conditions of low liquid volume or excessive power, ECs heating elements may exceed 300 °C, potentially producing irritant carbonyls such as formaldehyde and acrolein, thereby increasing the risk of chemical reactions that enhance toxicity.[14,18-20]

These differences in temperature, oxygen availability, and combustion explain the observed lower production of toxic species in ECs compared to conventional cigarettes, resulting in distinct emission profiles and toxicity levels.[16,19,20] In light of the growing use of EC products and their ever-changing chemical formulations, it is crucial to keep investigating their composition and behavior to inform regulatory frameworks and protect public health.

Despite existing regulations in some countries limiting nicotine concentrations in e-liquids, the lack of comprehensive standards for other potentially harmful substances remains a significant concern.[2,21]

In this context, it is imperative to conduct comparative investigations examining the chemical differences between combustible tobacco smoke and EC aerosols.[16] Only through rigorous scientific characterization the true risks of ECs can be elucidated and their role in harm reduction strategies be accurately assessed.[21]

METHODOLOGY

This study aimed to identify the presence of five target substances: diacetyl, benzaldehyde, acetaldehyde, formaldehyde, and VEA. Analyses were performed on 20 e-liquid samples and were categorized by compound type as follows: diacetyl analysis (20 samples), aldehyde analysis (benzaldehyde, acetaldehyde, formaldehyde; 20 samples), and vitamin E acetate analysis (20 samples). This study was approved for publication by the International School of Medicine within Universidad Anáhuac Cancún. The Research Committee of our School thoroughly reviewed the protocol, methodology, and content of the manuscript, determining that it meets the ethical and academic standards required. Consequently, the committee granted its approval and authorized the study's dissemination as part of the university's scientific output.

Analysis of Diacetyl and Aldehydes

Samples were prepared in 5 mL glass vials at different concentrations, sealed with PTFE-lined caps, and placed in 500 mL beakers containing water in a controlled-temperature water bath. The samples were heated to 80°C for 10 minutes to ensure temperature equilibration. Once stabilized, 0.6 mL aliquots were drawn using syringes and injected into the gas chromatography column. The compounds were analyzed using Headspace Gas Chromatography with Flame Ionization Detection (HS-GC-FID) and GC-MS, employing a Perkin Elmer Clarus 580 system with a Clarus SQ 8S mass spectrometer and a Perkin Elmer Autosystem GC with an FID detector. Volatile components were also extracted using a chloroform/water partitioning method, with diacetyl quantified from the aqueous phase. Flavor compounds were specifically analyzed via HS-GC-FID.

Identification Using Pure Standards

All sample preparations followed the same procedure, with identification of analytes based on retention time and molecular mass compared to pure analytical standards. For GC-MS analysis, 5 mL of each sample was subjected to liquid–liquid extraction using 10 mL of a water–chloromethane mixture (CH_3Cl, 1:1 v/v) in a separatory funnel, agitated three times for 10 minutes using a magnetic stir bar. The extracts were then dried in a vacuum oven at 40°C, concentrated to 1 mL, and a sample of 2 µL was injected into the GC-MS system.

Analysis of Vitamin E Acetate

For VEA analysis, 100 µL of each sample was combined with 2 mL of a pyridine–acetic anhydride solution (2:1 ratio) and left at room temperature for 24 hours until the reagents had fully evaporated. The dry residues were dissolved in 500 µL of chloroform, and 2 µL was injected into the GC-MS for analysis.

Analysis of Combustible Cigarette Smoke

A dual vacuum-trap system was assembled using two 125 mL flat-bottom round flasks connected by glass tubing. Each flask contained 30 mL of a methanol–dichloromethane solution (5:1 ratio) to adsorb smoke and volatile emissions from burning cigarettes.

Direct Analysis of 20 Combustible Cigarettes

Cigarettes were placed one by one into the manifold inlet and ignited; each was allowed to burn for 5 minutes while the resulting smoke passed through the vacuum-trap system. This process continued until all 20 cigarettes from a single pack were consumed. The trapping solvents were then evaporated, the residue was reconstituted in 1 mL of methanol–dichloromethane, and 5 µL was injected into the GC-MS for compound identification.

RESULTS

Four e-liquid samples (two domestic and two imported brands) were analyzed for the presence of five target compounds: diacetyl, formaldehyde, acetaldehyde, benzaldehyde, and VEA, in comparison with combustible cigarette smoke. A qualitative analysis was performed on each e-liquid using pure standards to screen for the presence of the target analytes. Based on molecular weight and retention time data, none of the analyzed e-liquids showed detectable traces of the target compounds when assessed via GC-MS. Specifically, diacetyl, formaldehyde, acetaldehyde, benzaldehyde, and VEA were not identified in any of the samples.

Table 1 exhibit the 27 most frequently identified compounds in combustible cigarette smoke, as detected through GC-MS following collection in a methanol–dichloromethane solution. The table includes compound names, CAS numbers, functional group classification, and toxicological classification (toxic and/or carcinogenic) according to the HSDB and the GHS. Chemically, the most prevalent compounds in cigarette smoke included aldehydes, ketones, aromatic hydrocarbons, and nitrogen-containing substances—byproducts characteristic of the pyrolysis of tobacco, paper, and additives. These compounds are largely generated through thermal degradation reactions, particularly at temperatures exceeding 600°C, where incomplete oxidation dominates.

As mentioned earlier, combustion in conventional cigarettes results in elevated levels of thermal degradation products such as formaldehyde, acrolein, acetone, benzene, naphthalene, and nitrogenated derivatives. From Table 1, it

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can be inferred that 10.2% of the compounds detected in cigarette smoke were classified as carcinogenic, including benzene, 1,3-butadiene, and several polycyclic aromatic hydrocarbons (PAHs), while 12.7% were classified as toxic, including respiratory irritants and sensitizing agents. Table 1 thus provides a critical reference point for toxicological comparison with compounds found in e-liquids. The higher prevalence of hazardous substances in combustible cigarette smoke supports the hypothesis that tobacco combustion is a major source of toxicant exposure relative to the aerosol emissions produced by vaporizers.

Table 1. Most Frequently Identified Compounds in Combustible Cigarette Smoke

TYPE	COMPOUNDS / DERIVATIVES	SYMPTOMS	CARCINOGENIC
Fatty Acids	Linoleic Acid	Irritation of respiratory tract, eyes, skin; nausea and vomiting.	✗
Fatty Acids	Nonadecanoic Acid	Skin irritation and gastrointestinal symptoms; cause of colorectal cancer.	✓
Fatty Acids	Alpha-Linolenic Acid	Respiratory mucosal irritation, blurred vision, cough, gastrointestinal symptoms.	✗
Alkaloid	Nicotine	Addictive substance; in the CNS it produces a feeling of well-being and relaxation. Severe intoxication causes gastrointestinal symptoms, respiratory secretions and bradycardia; teratogenic.	✗
Alcohol	Cyclopentanol	Irritation of respiratory mucosa, pulmonary edema, pneumonitis, hemorrhage; dehydration and dermatitis.	*
Alkyl Alcohol	5-Hexen-2-ol, 5-methyl	Irritation of respiratory mucosa, skin and gastrointestinal symptoms.	*
Fatty Alcohol	Behenyl Alcohol	Irritation of respiratory mucosa, CNS depression and low-grade hepatic effects.	✗
Terpenoid Alcohol	Geraniol	Allergic dermatitis, CNS depression, spastic paralysis and changes in liver weight.	✗
Terpenoid Aldehyde	Farnesal	Tearing, redness, swelling and blurred vision; allergic dermatitis and skin rash.	✗
Amides	N-Benzoyl-dl-alanine	Respiratory mucosal irritation, specific pulmonary toxicity.	*
Bromates	Carbromal	Mental confusion, ataxia, areflexia, loss of pupillary response, cyanosis, coma, pulmonary shock and disseminated intravascular coagulation defect.	✓
Aromatic Compound	Caryophyllene Oxide	Lung, brain, breast and liver neoplasms. (NOT LISTED BY IARC)	*
Polyprenyl Compounds	Vitamin E / Alpha-Tocopherol	Irritation of respiratory mucous membranes, nausea, headache and weakness.	✗
Saturated Alkane Hydrocarbon Ester	Octadecane, 1-(ethenyloxy)	Allergic dermatitis.	✗
Fatty Acid Esters	Ethyl Linoleate	Irritation of respiratory mucous membranes. Breast cancer.	✓
Fatty Acid Esters	Farnesyl Acetate	Irritation of respiratory mucous membranes.	✗
Steroid	α-Cholest-5-ene, 3-methoxy	Irritation of respiratory mucous membranes.	✗
Hydrazone (–C=N–NH_2)	4-Hydrazono-5-hydroxyimino-4,5,6,7-tetrahydrobenzofurazone	Irritation of respiratory, skin and eye mucous membranes; allergic reactions and gastrointestinal symptoms. Hepatocellular carcinoma, malignant bronchial and lung neoplasms.	✓
Benzenic Hydrocarbon	Cis-β-Caryophyllene	Acute myeloid leukemia. Irritation of respiratory mucous membranes.	✓
Saturated Alkane Hydrocarbon	Heptacosane	Irritation of respiratory mucous membranes. Squamous-cell carcinoma.	✓
Saturated Alkane Hydrocarbon	Tetratetracontane	Skin and eye irritation; gastrointestinal symptoms. Squamous-cell carcinoma.	✓
Alkyl Nitrates	Amyl Nitrate	Acute pulmonary edema, renal tubule alterations; inhalation causes convulsions, cyanosis, headache, methemoglobinemia and nausea.	*
Hydrocarbons	Benzenes	Leukemia; dizziness, excitement, pallor followed by flushing, weakness, headache, dyspnea, chest tightness, nausea and vomiting. Coma and possible death.	✓
Aldehydes	Formaldehyde	Respiratory irritation, cough, wheezing, asthma, pulmonary edema, lung and nasopharyngeal cancer, leukemia.	✓
Haloalkanes	Vinyl Chloride	Inhalation causes dizziness, CNS depression, hepatic cirrhosis, pulmonary irritation.	✓
Organochlorine Compounds	Dioxins	Eye irritation, allergic dermatitis, acne, gastrointestinal symptoms, teratogenic effects; possible hepatic and renal damage.	✓
Inorganic Acids	Sulfuric Acid	Respiratory tract irritation, tracheobronchitis, stomatitis, gastritis, gastric perforation, peritonitis, circulatory shock. Laryngeal and lung cancer.	✓
*	NOTE		
	No conclusive data indicate carcinogenicity; however, at high temperatures it may pose a potential toxic risk to pulmonary health.		

This table summarizes the 27 most frequently identified compounds found in the smoke of combustible cigarettes, based on gas chromatography–mass spectrometry (GC-MS) analysis. Compounds are classified by type, laboratory of origin, functional group, known health effects, and toxicological status according to the Hazardous Substances Data Bank (HSDB) and the Globally Harmonized System (GHS). The presence of a high proportion of carcinogenic and toxic substances in cigarette smoke highlights the significant health burden associated with tobacco combustion.

Table 2 lists the 24 most frequently identified compounds in the analyzed e-liquids, also determined by GC-MS. Each compound is detailed with its CAS number, functional group classification (e.g., aldehydes, ketones, esters), and toxicological classification as per HSDB and GHS criteria. From a chemical standpoint, Table 2 highlights the structural diversity of components present in commercial e-liquid formulations. Most of the compounds identified were low-molecular-weight volatile substances, many of which were esters and aldehydes derived from food-grade flavorings, along with fatty and nitrogenous compounds typically used as carriers (e.g., glycerin, propylene glycol) or to enhance sensory profiles.

Table 2. Most Frequently Identified Compounds in Analyzed E-Liquid Samples

TYPE	COMPOUNDS / DERIVATIVES	SAMPLE 1 - 0 MG	SAMPLE 1 - 3 MG	SAMPLE 1 - 6 MG	SAMPLE 2 - 3 MG	SAMPLE 3 - 3 MG	SAMPLE 4 - 0 MG	SAMPLE 4 - 3 MG	SAMPLE 5 - 0 MG	SAMPLE 5 - 3 MG	COMMON USES	SYMPTOMS	CARCINOGENIC
Esters	Ethyl citrate	✓	✓	✓	✓	✓	✓		✓	✓	In foods as a citrus-flavouring agent, solvent and surfactant.	Irritation and/or sensitivity of mucous membranes, cough, vomiting. Causes metastasis in stromal tumours.	✓
Esters	Ethyl acetate		✓			✓		✓	✓		Printing inks, paint thinners/solvents, textile cleaners, perfume manufacture, solvent for explosive compounds.	Headache, nausea, loss of consciousness.	✗
Esters	Nona-lactone (Coconut lactone)	✓	✓	✓							Coconut/fruit flavouring, cleaning products, cosmetics, perfumery.	Irritant effects on the respiratory tract.	✗
Carboxylic acids	Stearic acid, methyl ester	✓	✓	✓			✓				Stable base for lotions, creams and deodorants.	Respiratory-tract irritation, gastrointestinal disorders.	✗
Carboxylic acids	Palmitic acid, ethyl ester	✓		✓	✓					✓	Soaps, detergents and cosmetics.	Increased risk of cardiovascular disease and breast cancer in post-menopausal women.	✓
Carboxylic acids	Di-n-octyl phthalate						✓			✓	Production of resins, plastics, dyes, pharmaceuticals and fungicides.	Cough, dyspnea, wheezing, liver damage, systemic toxicity.	✗
Carboxylic acids	Adipic acid, diethyl ester			✓	✓		✓				Nylon production, manufacture of clothing, tires and carpets.	Cough, odynophagia.	✗
Carboxylic acids	2-Decenoic acid, methyl ester	✓	✓				✓				Food preservatives.	Headache, nasal mucosa irritation, liver and kidney involvement.	*
Aromatic aldehydes	4-Acetyloxy-3-methoxybenzaldehyde	✓		✓			✓	✓			Flavouring (anise/almond).	Cough, headache, nausea, vomiting.	*
Aromatic aldehydes	Isovanillin	✓		✓			✓	✓			Vanilla- or caramel-like flavouring.	Respiratory toxicity, irritation and pulmonary inflammation.	*
Aromatic aldehydes	Vanillin	✓	✓	✓			✓	✓			Cosmetics, perfumes, flavouring.	Not classified as toxic.	✗
Flavourings	Ethyl maltol	✓	✓	✓	✓		✓	✓	✓	✓	Food additive; enhances sweet caramel flavours.	Hepatic and renal damage. Oncogenic when combined with metals.	✓
Flavourings	Maltol	✓	✓	✓			✓				Flavour enhancer.	Eyes: tearing, swelling, redness, blurred vision. Respiratory: mucosal irritation.	*
Alcohols & polyols	Glycerin	✓	✓	✓	✓		✓		✓		Cosmetics, food preservative, sweetener.	Mucosal irritation, cough and wheezing.	*
Alcohols & polyols	Propylene glycol	✓	✓	✓	✓		✓		✓		Cosmetics, pharmaceuticals, antifreeze additive (cooling systems).	Headache, nasal mucosa irritation, liver and kidney damage.	*
Nitrogen compounds	1-Nitroso-azetidine		✓			✓		✓	✓		Chemical industry.	Tumorigenic agent affecting gastrointestinal system, liver and thorax.	✓
Nitrogen compounds	Methylamine				✓		✓	✓			Agrochemicals (herbicides, fungicides, insecticides, biocides, acaricides); fuel additive.	Irritation of nasal/oropharyngeal mucosa, cough, wheezing. Repeated exposure may cause bronchitis, breathing difficulty, liver effects.	*
Nitrogen compounds	Guanosine	✓	✓	✓			✓	✓	✓	✓	Manufacture of certain pharmaceuticals.	Vomiting, nausea.	✗

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Nitrogen compounds	Pyridine (3-[1-methyl-2-pyrrolidinyl]-, (S)-)	✓	✓	✓	✓	✓	✓	✓		✓	Solvent; manufacture of medicines, vitamins, food flavourings, pesticides, paints, dyes, rubber products, adhesives and waterproofing for fabrics.	Mucosal irritation, headache, nausea, respiratory difficulty, cardiovascular diseases.	✗
Others	Fluoxymesterone (synthetic steroid)									✓	Chemical & pharmaceutical industry (hormone-replacement therapy).	Acne, virilization, fluid retention, hyperkalemia; teratogenic in animals.	*
Others	Vulcanol (2,4,7,9-Tetramethyl-5-decyn-4,7-diol)	✓	✓	✓		✓		✓			Raw material for plastics, adhesives, construction materials, paints, solvents; low-emission fuel.	Dryness of nasal and oropharyngeal mucosa, epistaxis.	✗
Others	Triamcinolone acetonide (corticosteroid)									✓	Pharmaceutical industry to treat inflammatory, atopic and allergic conditions.	Pulmonary damage at uncontrolled doses; immune-system suppression.	*
Others	Alpha-carotene		✓	✓		✓			✓	✓	Pharmaceutical industry; adds yellow/orange colour.	Irritability, loss of appetite and weight, fever, pulmonary involvement (pneumoconiosis).	✗
Others	1,4-Dioxane (stabilising ether)		✓			✓		✓	✓		Chemical & pharmaceutical industry; solvent for cellulose acetate, resins, oils, waxes, dyes and other compounds.	Respiratory-tract mucosal irritation, cough, dyspnoea, dizziness, headache. Nasopharyngeal and hepatic cancer.	✓
*	NOTE												
	No conclusive data indicate carcinogenicity; however, at high temperatures it may pose a potential toxic risk to pulmonary health.												

This table presents the 24 most frequently identified compounds in commercial e-liquid formulations, analyzed via gas chromatography–mass spectrometry (GC-MS). Each compound is categorized by type, functional group, and known health effects, including toxicological classification according to the Hazardous Substances Data Bank (HSDB) and the Globally Harmonized System (GHS). While structurally diverse, the compounds detected in e-liquids showed a notably lower proportion of carcinogenic and toxic agents compared to those found in combustible cigarette smoke, reinforcing the hypothesis of reduced chemical risk in non-combustion nicotine delivery systems.

A key finding is that, despite the chemical complexity of the mixtures, only 1.7% of the compounds detected in e-liquids were classified as carcinogenic, and 5.5% as toxic, representing a considerably lower toxicological burden than that of combustible cigarette smoke. Among the substances with adverse toxicological classification were certain aldehydes with irritant potential and esters that may thermally degrade into hazardous byproducts. However, none of the most critical compounds —formaldehyde, acetaldehyde, or diacetyl— were identified in detectable concentrations.

Discussion

In the present study, we identified and characterized toxicologically relevant chemical compounds in selected domestic and international commercial e-liquids. Five key analytes were analyzed: diacetyl, formaldehyde, acetaldehyde, benzaldehyde and vitamin E acetate (VEA). Additionally, we compared the chemical composition of these e-liquids with that of combustible cigarette smoke. To ensure analytical reliability, we applied robust chromatographic techniques —HS-GC-FID and GC-MS— with standardized sample preparation and validation using pure standards. This methodological rigor enhanced confidence in compound identification.

As previously mentioned, across all 20 e-liquid samples analyzed, none showed detectable levels of diacetyl, formaldehyde, acetaldehyde, benzaldehyde, or VEA, based on the methods employed. However, while the number of total compounds detected in e-liquids was broad, the proportion

of hazardous substances was considerably lower than that found in combustible cigarette smoke: only 1.7% were classified as carcinogenic and 5.5% as toxic, according to the HSDB. Conversely, the analysis of combustible cigarette smoke under standardized conditions (absorption in a methanol–dichloromethane mixture and controlled evaporation) revealed the presence of compounds classified as potentially carcinogenic and toxic according to the GHS and HSDB. Specifically, 10.2% were identified as carcinogenic and 12.7% as toxic.

Both matrices —e-liquids and cigarette smoke— contained aldehydes, ketones, esters, nitrogenated compounds, and fatty acids. However, combustible cigarette smoke exhibited a greater proportion of thermal degradation products and byproducts of incomplete combustion, including free radicals and polycyclic aromatic hydrocarbons (PAHs), which are well-established contributors to tobacco-related toxicity.[22] The combination of HS-GC-FID and GC-MS techniques used in this study ensures high validity of these comparative results. As previously discussed, electronic devices operate at much lower temperatures (typically ~250°C) compared to lit cigarettes, which can reach peaks of up to 950°C.[23] These temperature differences, along with the absence of combustion and tobacco in e-cigarettes, are key factors in the substantially lower levels of toxicants in EC aerosols compared to cigarette smoke.[24]

The comparative findings from this study reinforce the well-established notion that, while not harmless, e-liquids and e-cigarette aerosols pose a lower overall toxicological burden than conventional tobacco smoke.[25] Other studies have similarly reported the presence of toxicants in e-cigarette vapors, though generally at levels 9 to 450 times lower than those found in combustible cigarette smoke, and often comparable to background or trace levels.[26]

This comparatively less hazardous chemical profile can be partly attributed to the absence of combustion processes in ECs, which restricts the formation of additional hazardous by-products. Additionally, many components are derived from the food industry and are generally recognized as safe (GRAS) for oral use. However, safety via inhalation does not necessarily equate to oral safety, which highlights the need for future studies in this field.

Table 2 provides a representative chemical characterization of commercial e-liquids and supports the hypothesis that, although not entirely risk-free, these products exhibit a significantly lower toxicological profile compared to tobacco combustion products.

Numerous researchers have analyzed the chemical compositions of e-liquids and aerosols, including commercial and prototype products, through systematic reviews aimed at understanding their potential health risks and benefits.[21] In April 2024, the Royal College of Physicians (UK) published a report based on a systematic review of studies exploring the relationship between vaping, toxicant exposure, and biomarkers of potential health damage. The report concluded that, compared to smokers, vapers are exposed to a much narrower and lower spectrum of toxicants (as shown in the present work), including reduced carbon monoxide levels. Moreover, it noted that nicotine vaping is not associated with a high frequency of adverse health outcomes, especially when adjusted for prior smoking history.[27]

In line with these findings, the methodological framework of this study, which included aqueous/organic phase separation for the detection and identification of volatile compounds based on molecular mass and retention time, provided a robust and reproducible basis for evaluating the chemical profiles of e-liquids and combustible cigarette smoke. The results support the hypothesis that the analyzed e-liquids may represent a lower-risk alternative to combustible cigarettes in the short to medium term.[25] However, this relative advantage should be interpreted with caution.

We argue that chronic inhalation of complex chemical mixtures, even in the absence of traditionally regulated substances, may pose long-term health risks that remain insufficiently understood and warrant further investigation. In our view, the findings presented here raise important concerns regarding formulation transparency and underscore the urgent need for regulatory standards on the composition and inhalation safety of these products.

Study Limitations

Although this study employed robust analytical techniques such as HS-GC-FID and GC-MS, several limitations must be considered when interpreting the findings:

Restricted scope of target compounds

The analysis was limited to five specific substances (diacetyl, formaldehyde, acetaldehyde, benzaldehyde, and VEA). While these compounds are well-documented for their toxicological relevance, the complex chemical composition of e-liquids suggests that additional contaminants or degradation products —potentially present— were not evaluated. The dynamic nature of commercial formulations also contributes to substantial variability in product composition across the market.

Qualitative rather than quantitative analysis

The methodology focused on compound identification based on retention time and molecular mass relative to pure standards. However, no quantitative determination was performed using calibration curves or validated limits of detection (LOD) and quantification (LOQ), thereby limiting the precision of residual concentration assessments. Moreover, this methodological and qualitative approach prevented statistical comparison of the samples, as the broad list of compounds was obtained using different protocols and at different time points. To enable quantitative comparison, it would be necessary to prepare e-liquid samples from different brands using the same flavor and identical nicotine concentrations to ensure consistent experimental conditions. This type of analysis would be both interesting and necessary for future studies.

Limited sample representativeness

A total of 20 e-liquid samples (10 domestic, 10 international) and one brand of combustible cigarette were analyzed. While sufficient for preliminary comparison, this sample size does not fully represent the breadth of vaping products or the diversity of combustible tobacco brands available, limiting the generalizability of results.

Limited detection of thermally unstable or low-volatility compounds

HS-GC-FID is well-suited for detecting small, moderately volatile molecules such as aldehydes. However, it may fail to identify thermally labile, low-volatile, or highly polar compounds—including oxidized glycols, cannabinoids, pesticides, or high-molecular-weight components—commonly found in some e-liquids. These substances might remain undetected unless complemented with techniques such as LC-MS or non-volatile residue analysis.

Absence of aerosol analysis under real-use conditions

The study did not analyze aerosols generated during active inhalation or under realistic vaping conditions, which could yield different chemical profiles due to thermal transformation during use.

Lack of standardization in sensory and flavor profile characterization

No structured assessment of the flavoring and sensory components was included, despite their potential to influence user exposure and chemical reactivity during vaporization. Therefore, while this study provides a useful comparative chemical characterization between e-liquids and combustible cigarettes, additional research is required. This should include quantitative analyses, real-use simulations, broader sample populations, and complementary toxicological studies to achieve a more comprehensive risk assessment.

It is also important to note that laboratory vaporization settings do not fully replicate real-world user behavior. Variables such as device power output, coil temperature, puff volume, and usage frequency can dramatically influence the formation of thermal degradation products. While controlled temperatures were used in this study, they may not account for overheating scenarios commonly encountered during daily use.

CONCLUSION

Based on the number and classification of carcinogenic and potentially toxic compounds detected via gas chromatography, the findings of this study suggest that the chemical constituents of the analyzed e-liquids may pose a lower health risk compared to those of combustible cigarettes. Nevertheless, e-liquids are not free from health risks. Future research is needed to deepen our understanding of the health effects associated with vaporized e-liquids and to clarify their potential role in harm reduction strategies.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this research. None of the participants have received financial, professional, academic, or personal benefits that could have influenced the results or interpretation of the study. Dr. Christian Heinrich-Henonin is an active medical writer and consultant for Philip Morris International (PMI); however, this affiliation did not influence the design, execution, analysis, or conclusions of the present study.

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Original research

In vitro comparison of two antimicrobial pastes used in pediatric pulp treatment against Enterococcus faecalis ATCC 51299

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ABSTRACT

Introduction: Successful pulp treatment is achieved with proper disinfection of root canals, which is difficult to achieve completely in primary teeth due to the complex anatomy. Antimicrobial pastes can be an effective treatment alternative, reducing work time and material use. **Methods**: Reference strain *Enterococcus faecalis* ATCC 51299 was used. Bacterial suspensions adjusted to 0.5 McFarland standard were incubated for 24 h at 37°C with both pastes. Serial dilutions (10-1-10^{-12}) were done, and colony-forming units (CFU/ml) were determined. **Results**: Both pastes inhibited bacterial growth, with statistically significant differences ($p<0.0001$). The CTZ (LAB) paste demonstrated greater antimicrobial effectiveness compared to the CTZ(UNAM) paste and the Ultrapex® paste. **Conclusions:** CTZ and Ultrapex® pastes demonstrated antimicrobial activity against *E. faecalis*, with CTZ being more effective. CTZ represents a promising alternative for pulpal treatment in primary teeth.

Key words: CTZ paste; Ultrapex®; *Enterococcus faecalis*; antimicrobial activity; pediatric endodontics; primary teeth.

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RESUMEN

Introducción: El tratamiento pulpar exitoso se logra con una desinfección adecuada de los conductos radiculares, lo cual en dientes temporales es difícil de lograr en su totalidad debido a la compleja anatomía. Las pastas antimicrobianas pueden ser una alternativa de tratamiento eficaz, reduciendo tiempos de trabajo y material utilizado.**Objetivo:** Evaluar la actividad antimicrobiana "*in vitro*" de la pasta CTZ y Ultrapex® sobre *Enterococcus faecalis*. **Métodos:** Se utilizó la cepa de referencia *Enterococcus faecalis* ATCC 51299. Se prepararon suspensiones bacterianas ajustadas a la escala de 0.5 de McFarland. Las suspensiones fueron incubadas por 24 h a 37 °C en presencia de ambas pastas. Se realizaron diluciones seriadas (10-1- 10^{-12}) y se determinó el número de unidades formadoras de colonias (UFC/mL). **Resultados:** Ambas pastas mostraron inhibición del crecimiento bacteriano, con diferencias estadísticamente significativas ($p < 0.0001$). La pasta CTZ (LAB) presentó una mayor eficacia antimicrobiana en comparación con la pasta CTZ (UNAM) y la pasta Ultrapex ®. **Conclusiones:** La pasta CTZ y la pasta Ultrapex® demostraron actividad antimicrobiana contra *E. faecalis*, siendo CTZ más efectiva. Su uso representa una alternativa viable en tratamientos pulpares de dientes temporales.

Palabras clave: pasta CTZ, ultrapex®; *Enterococcus faecalis*; actividad antimicrobiana; endodoncia pediátrica; dientes temporales.

INTRODUCTION

Pulpal disease in primary teeth represents a frequent clinical challenge in pediatric dentistry, largely due to the susceptibility of the dentin-pulp complex to injury. Dental caries is defined as a demineralization process of the tooth enamel, resulting from an ecological imbalance of the microorganisms present in the biofilm on the tooth surface. An increase in the prevalence of carious lesions has been observed in the pediatric population of Mexico, often leading to irreversible pulp involvement.[1,2,3]

Microorganisms most frequently associated with pulp lesions in primary teeth are *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp., *Peptostreptococcus* spp., *Streptococcus intermedius*, *Enterococcus faecalis*, *Clostridium* spp., and *Actinomyces israelii*, all of which are commonly implicated in irreversible pulpitis, pulp necrosis, and periapical abscesses.[4,5,6] Within this group, the genus *Enterococcus* is of particular clinical relevance with *E. faecalis* being the most frequently isolated species and is strongly associated with endodontic treatment failure due to its high resistance to intracanal medications.[7,8]

Factors explaining the high levels of resistance of *E. faecalis* includes the formation of dense and well-organized biofilms that hinder the penetration of antimicrobial agents, as well as its ability to invade and colonize dentinal tubules, where it remains protected from irrigation and environmental changes. Furthermore, *E. faecalis* can survive in nutrient-limited conditions and tolerate extreme pH variations, including the alkaline levels induced by calcium hydroxide, which enhances its persistence in endodontic failure.[7,8]

Pulp treatment remains essential for preserving primary teeth, although complete disinfection is challenging due to their complex anatomy.[4,9-11] Calcium hydroxide-iodoform pastes are the most widely used obturation materials in pediatric dentistry.[12] Commercial products such as Vitapex®, Metapex®, and Ultrapex® are considered ideal for filling primary canals.[13] However, these materials can undergo rapid resorption —compromising apical sealing— along with progressive loss of radiopacity and limited antimicrobial efficacy against certain bacterial genera.[11,12]

In Japan, a NO- instrumentation endodontic technique using antibiotic pastes —particularly CTZ, composed of chloramphenicol, tetracycline, and zinc oxide-eugenol— was developed for pulp disinfection[8,15] Although simple, low-cost, and broad-spectrum, it presents drawbacks such as crown discoloration, variable formulation quality and limited long-term evidence with recommendations to avoid its use in children under three years.[15] Guedes et al., (2006) further demonstrated its antimicrobial potential, reporting that CTZ produced the largest inhibition halos against reference strains commonly associated with pulp necrosis, while Vitapex®, showed no activity.[16]

At the Dental Specialty Unit (DEFENSA), a considerable proportion of paediatric patients require emergency care due to pain and infection. Notwithstanding the ongoing efforts to prevent such complications, a significant proportion of patients present with irreversible or necrotic pulpal conditions. These cases frequently arise within stringent time constraints, rendering antimicrobial pastes a pragmatic therapeutic option as they curtail treatment time and instrumentation demands.

The utilisation of CTZ paste as an alternative in pulpectomy procedures for primary teeth has demonstrated favourable clinical outcomes. However, despite the documented antimicrobial properties of both CTZ and Ultrapex®, the paucity of direct comparative in vitro evidence underscores the necessity for further investigation to ascertain their relative efficacies. The objective of the present study was to compare the in vitro antimicrobial activity of CTZ paste and Ultrapex® against *E. faecalis* ATCC 51299 to determine whether statistically significant differences exist between both formulations under standardised laboratory conditions.

Materials and Methods

An experimental, comparative in vitro study was conducted using the *E. faecalis* ATCC 51299 strain, which was provided by the Bacteriology Laboratory strain bank at the National School of Biological Sciences (IPN). For the microbiological assays, Müeller-Hinton® (MH) medium was utilised for both agar plates and liquid media, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (2024).

Preparation of the bacterial strain

The reference strain was thawed and streaked on 5% blood agar using the cross-streak technique. Subsequently, the plates were subjected to an incubation process at a temperature of 37°C for a duration of 24 hours. The viability and purity of the sample were confirmed through conventional tests, including Gram staining, catalase, and oxidase, among others. Cryovials were prepared for the preservation of the strain at a temperature of −70°C.

Preparation of the pastes

The paste assays were performed using approximately 30 mg, based on the commercial dosage indicated by the Ultrapex® syringe packaging. The specified amount of solution was dispensed into each well of a 24-well microplate.

CTZ paste was procured from the National Autonomous University of Mexico (UNAM). For its preparation, 30 mg of CTZ powder (UNAM) was meticulously weighed on a glass slab, and two drops of eugenol (approximately 100 µL) were added with the objective of achieving a semi-solid, homogeneous, non-sticky paste. This consistency description is applicable solely to the handling properties of the paste in clinical practice. In the present study, no root canals were inoculated; all evaluations were performed in vitro using 24-well microplates. Following the mixing process, the weight of each sample was once again verified using an analytical balance (Fisher Scientific®) prior to the inoculation of each well of the microplate.

Additionally, a CTZ (LAB) paste was prepared in the laboratory using each component based on the classical CTZ formulation in a 1:1:2 ratio (chloramphenicol, tetracycline, and zinc oxide). The paste was made by mixing 500 mg of generic tetracycline, 500 mg of chloramphenicol (PiSA®), 1000 mg of zinc oxide (Viarden®), and one drop of eugenol (approximately 50 µL). The components were then amalgamated on a glass slab using a metal spatula, continuing until a homogeneous, semi-solid, workable, and stable mixture was obtained, as previously described. Stock solutions were subsequently prepared at a concentration of 30 mg/mL.

Bacterial suspension and assay setup

The working bacterial suspension was adjusted in Müeller-Hinton (MH) broth to 0.5 on the McFarland scale, corresponding to approximately 1.5×10^8 CFU/mL (CLSI, 2024). The three formulations previously described were evaluated: Ultrapex®, CTZ (UNAM), and CTZ (LAB). In a 24-well microplate (Thermo Scientific®), the bacterial suspensions were distributed with each of the pastes (30 mg). The following controls were included: bacterial suspension without paste (growth control), culture medium alone (sterility control), and culture medium with paste (media interference control). This last control was included to assess whether the paste altered the culture medium conditions (e.g.,or pH, turbidity, precipitation) that could lead to non-specific inhibition or artificial bacterial proliferation. No alterations were detected, thereby confirming that the paste did not interfere with the medium under the experimental conditions or that any contamination was not observed during the manipulation. In order to ensure the reliability of the results, three replicates of each bacterial suspension, in addition to controls, were performed for each paste. The microplates were then subjected to an incubation process in a New Brunswick Scientific® shaking incubator, operating at a speed of 150 revolutions per minute and a temperature of 37 degrees Celsius, for a duration of 24 hours. Following a 24-hour period, serial dilutions were performed to ascertain the concentration of colony-forming units per millilitre (CFU/mL).

Determination of CFU/mL

To determine the number of colony-forming units per millilitre (CFU/mL), serial dilutions were prepared from an aliquot of the bacterial suspension (Müeller-Hinton) from each experimental well (containing bacteria and paste). Dilutions ranged from 10^{-1} to 10^{-12}.

For each experimental replicate, serial dilutions (10^{-10}, 10^{-11}, 10^{-12}), were plated in parallel on Müeller-Hinton agar in triplicate. Plates were incubated at 37°C for 24 hours, and CFU were counted from the dilution that yielded 30-300 colonies, in accordance with standard microbiological quantification criteria.

Statistical analysis

Prior to conducting the analysis of variance (ANOVA), the normality of the data was evaluated using the Shapiro-Wilk test. Subsequently, a multiple comparisons analysis was performed using Tukey's test, with the Epidat® statistical software employed for mean comparison. The objective of this study was to ascertain whether the variability observed in the arithmetic mean was attributable to random chance. A significance threshold of $p < 0.0001$ was established; therefore, values equal to or lower than this threshold were interpreted as statistically significant differences.

RESULTS

Bacterial Strain

Viability and purity of the strain was confirmed conventional tests, including Gram staining, catalase, and oxidase, among others (Figure 1). All the cryovials were prepared to preserve the strain at −70°C.

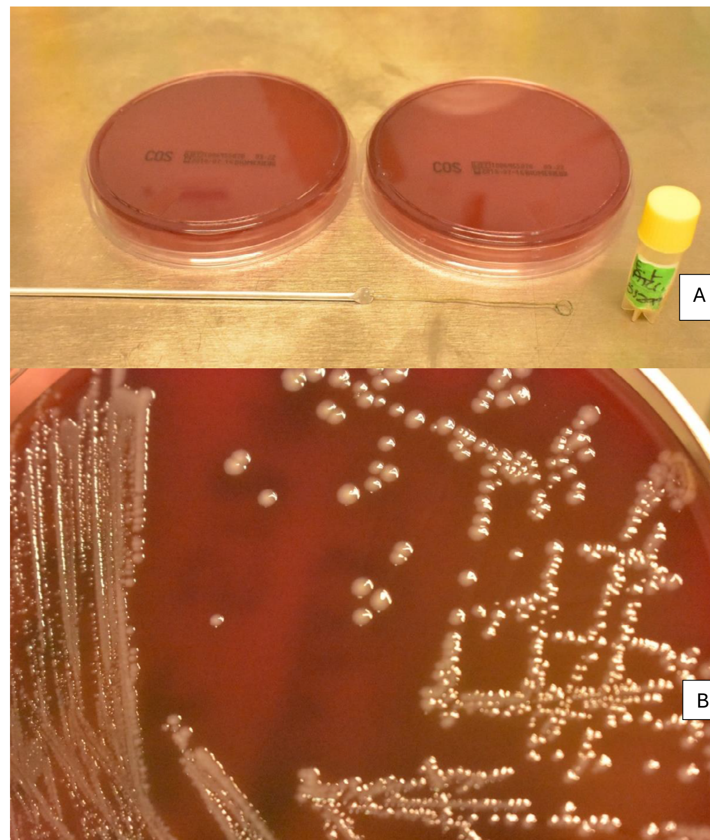

Figure 1. Viability and purity testing was performed on Casman agar (A). *Enterococcus faecalis* colonies are typically small, smooth, circular, and grayish white and non-hemolytic.

Preparation of the pastes

The CTZ (UNAM) paste was acquired from UNAM and it was prepared as previously described. The distribution of the assay in the 24-well microplate is observed in Figure 2.

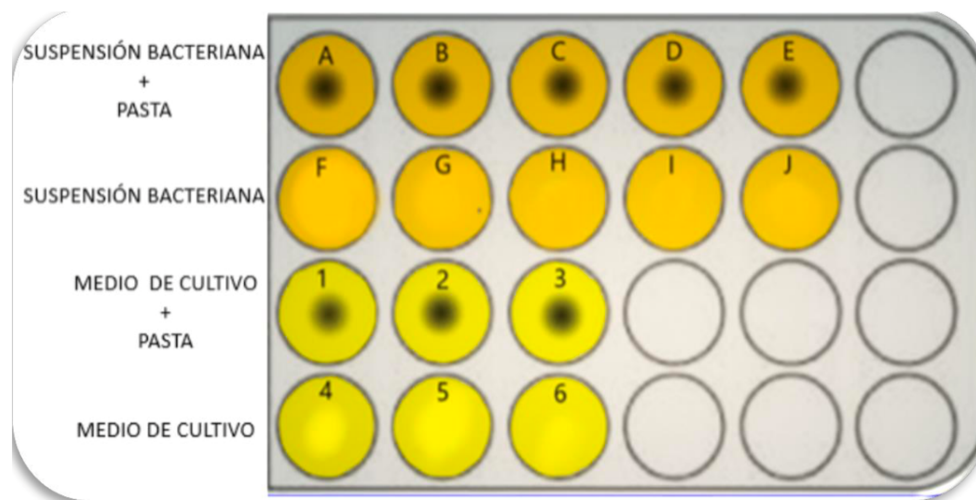

Figure 2. Distribution of the assays. Wells A-E contain bacterial suspension plus paste. Wells F-J contain bacterial suspension only. Control wells 1-3 contain culture medium plus paste. Control wells 4-6 contain culture medium only.

The initial assay performed with CTZ (UNAM) paste was not considered representative for analysis because the paste exhibited an oily separation that prevented homogeneous contact with the bacterial suspension (Figure 3). Due to the oily separation observed in the initial CTZ (UNAM) paste preparation, which prevented homogeneous contact with the bacterial suspension, the initial results were excluded and a pre-treatment was done.

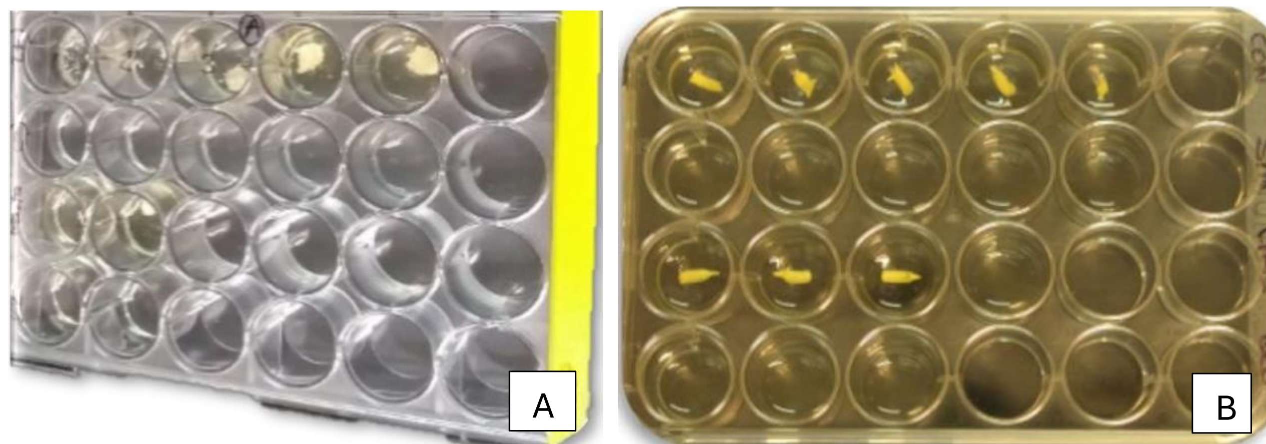

Figure 3. Distribution of the assays with (A) CTZ (UNAM) paste and (B) Ultrapex ® paste. A noticeable separation can be observed in the CTZ (UNAM) paste, which prevents homogeneous dispersion throughout the well. In contrast, Ultrapex ® exhibits uniform and consistent distribution in the bacterial suspension, allowing adequate contact between the paste and the culture medium.

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To standardize the experimental conditions between formulations, both pastes were prepared as previously described, spread on a glass slab and dehydrated in an incubator at 38 oC for 24 hours (Figure 4). However, the CTZ (LAB) paste was prepared directly from powdered components requiring only one drop of eugenol volume to achieve workable consistency, following the handling conditions recommended for this formulation.

Figure 4. CTZ(UNAM) paste mixture prior to the dehydration process.

The stock solutions were prepared by weighing 30 mg of the previously dehydrated powder using a Fisher Scientific A-250® analytical balance. The powders were then ground in an Omni™ tissue homogenizer in Eppendorf tubes with 1 mL of sterile PiSA® water (Figure 5). Sterile water was used following the CLSI 2024 guidelines for solvents and diluents in antimicrobial stock preparations for ensuring comparable consistency, solubility and direct contact conditions across all assays.[17]

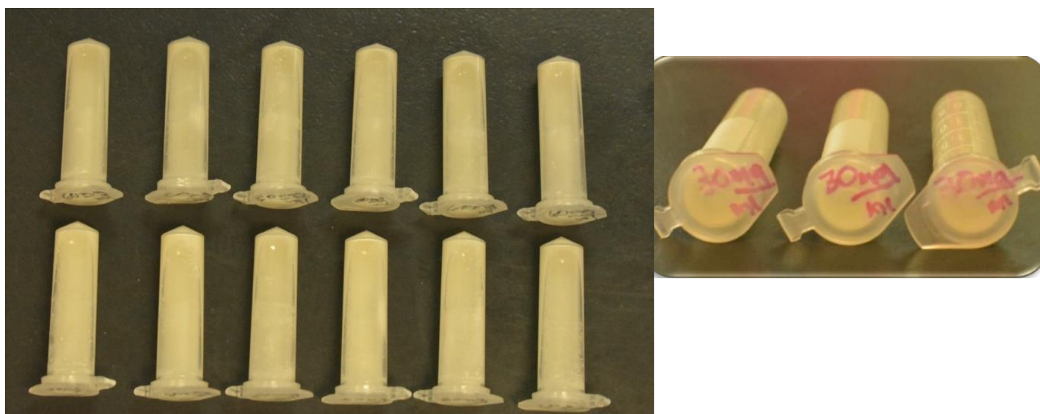

Figure 5. Stock solutions of 30 mg/mL of the CTZ paste.

Determination of CFU/mL

A second standardized assay was then performed. All reported results and statistical analyses in this study are based on this standardized phase (Figure 6). A dilution from the stocks were done according to CLSI (2024), since 30 mg is the Minimum Inhibitory Concentration (MIC) for chloramphenicol and tetracycline. This CMI represents the lowest concentration of an antimicrobial drug that prevents the visible growth of a microorganism in a laboratory setting.

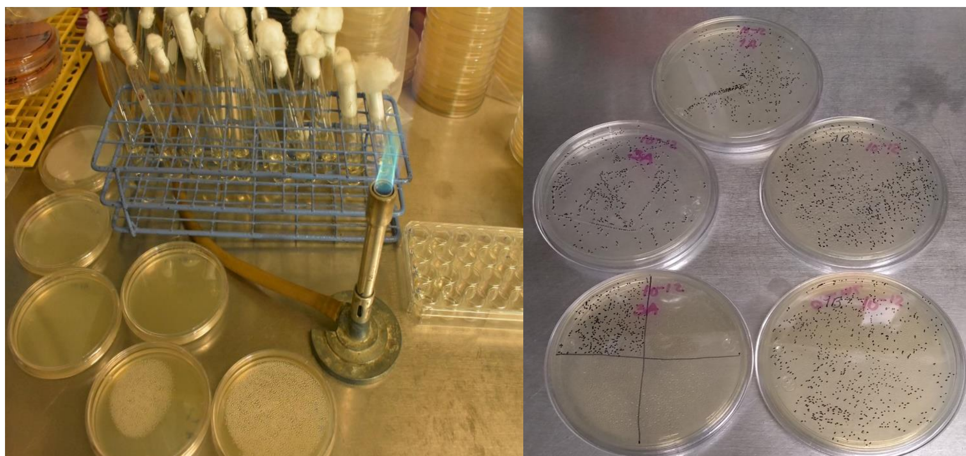

Figure 6. Material used for obtaining the CFU/mL. (A) Tubes used for serial dilutions and the corresponding MH agar plates are shown; (B) A representative image of the colonies obtained after the assay.

The colony counts obtained after the assay, compared to the control group, are shown in Table 1. The table presents the average values of colony-forming units per milliliter (CFU/mL) from the three replicates performed for each paste. The control group maintained a stable microbial load of 5.4×10^{18} CFU/mL in all assays.

Table 1. CFU/mL counts for *E. faecalis* with the different pastes

Paste	Assay 1 (avg)	Assay 2 (avg)	Assay 3 (avg)	Mean SD
	CFU/mL	CFU/mL	CFU/mL	CFU/mL
Growth control	5.4 X 1018	5.4 X 1018	5.4 X 1018	**$5.4 \times 10^{18} \pm 0$**
Ultrapex®	4.20 X 1016	5.33 X 1016	6.95 X 1016	**$5.49 \times 10^{16} \pm 1.38 \times 10^{16}$**
CTZ (UNAM) powder	5.4 X 1018	4.96 X 1018	6.4 X 1018	**$5.59 \times 10^{18} \pm 7.38 \times 10^{17}$**
CTZ (UNAM) paste	5.4 x1018	5.5 x1018	5.6 x1018	**$5.50 \times 10^{18} \pm 1.00 \times 10^{17}$**
CTZ (LAB) powder	8.7 x1015	2.47 x1016	1.92 x1016	**$1.75 \times 10^{16} \pm 8.13 \times 10^{15}$**
CTZ (LAB) paste	NG	NG	NG	NG

*NG. No bacterial growth observed.

Based on the CFU/mL quantification, the growth control maintained a stable bacterial load of 5.4×10^{18} CFU/mL across all assays. Ultrapex® demonstrated a reduction of approximately two logarithmic units compared to the control, with a mean value of $5.49 \times 10^{16} \pm 1.38 \times 10^{16}$ CFU/mL.

When performing the statistical analysis comparing the control group with CTZ (UNAM), both in powder and paste form, the resulting p-value was > 0.9999, indicating no statistically significant difference between them. Thus, neither formulation showed antimicrobial activity against the ATCC strain of *E. faecalis*.

In contrast, comparisons between the control vs. Ultrapex® and control vs. CTZ (UNAM) powder yielded $p < 0.0001$, indicating statistically significant differences. Therefore, Ultrapex® and the non-commercial CTZ paste demonstrated antimicrobial activity, reducing the bacterial count by two logarithmic units (Figure 7).

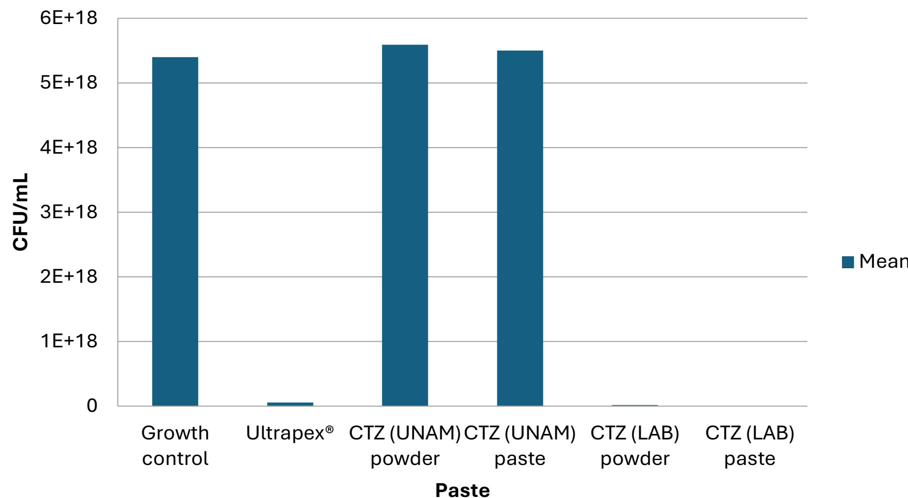

FIGURE 7. No significant differences were found between Control and CTZ (UNAM) powder or paste (p > 0.9999), confirming that neither CTZ (UNAM) formulation exhibited antimicrobial activity.

The bar graph indicates that the Growth control, CTZ (UNAM) powder, and CTZ (UNAM) paste demonstrated mean CFU/mL values around 10^{18}, with no discernible decrease in bacterial load. Conversely, Ultrapex® and CTZ (LAB) powder had significantly lower results, around 10^{16} CFU/mL, indicating a reduction of around two logarithmic units relative to the control. The CTZ (LAB) paste exhibited no observable bacterial growth, confirming total inhibition under the experimental circumstances.

The analysis of CFU/mL values revealed clear differences among the tested groups. The Control, CTZ (UNAM) powder, and CTZ (UNAM) paste groups showed bacterial counts within the 10^{18} range, with no statistically significant differences between them (Tukey, p > 0.9999) (Figure 8).

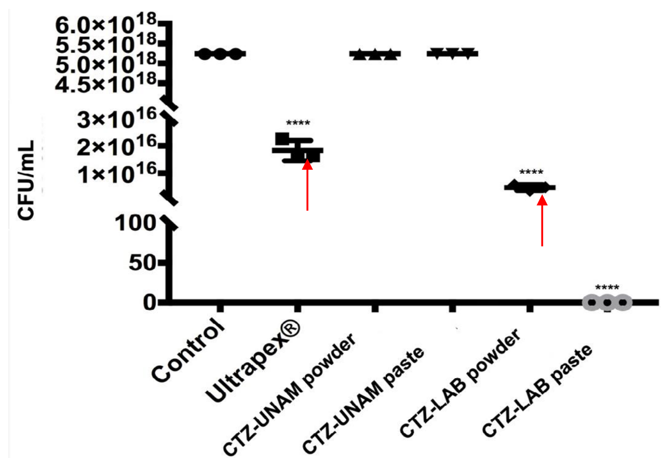

FIGURE 8. CFU/mL values of *E. faecalis* after exposure to the tested pastes (log scale). Control, CTZ (UNAM) powder, and CTZ (UNAM) paste showed comparable bacterial counts with no significant differences (Tukey, p > 0.9999).

Ultrapex® demonstrated a significant reduction in bacterial load to the 10^{16} range (Tukey, $p < 0.0001$), showing lower CFU/mL values compared with the Control and both CTZ (UNAM) formulations. Similarly, the CTZ (LAB) powder exhibited a significant decrease in CFU/mL (Tukey, $p < 0.0001$), while the CTZ (LAB) paste showed no detectable bacterial growth, differing significantly from all other groups (Tukey, $p < 0.0001$). Overall, these findings indicate statistically significant reductions for Ultrapex®, CTZ (LAB) powder, and CTZ (LAB) paste, whereas no reduction was observed for the CTZ (UNAM) formulations.

DISCUSSION

Diverse clinical studies have documented the efficacy of various materials employed in pulpal treatments of primary teeth. Nevertheless, the evidence from in vitro studies that consistently demonstrates the ability of these materials to inhibit bacterial growth remains limited.[18]

In the present study, the antimicrobial activity of the pastes was evaluated under in vitro conditions. An initial investigation was conducted to evaluate the antimicrobial properties of Ultrapex® paste. This investigation was conducted in accordance with the methodology established by Velasco (2012), in which filter paper disks were impregnated with Ultrapex®. In Velasco's study, the inhibition halos were described as minimal. In addition, no inhibition halos were observed in the laboratory, likely due to the specific properties of Ultrapex®'s composition, which does not diffuse well in agar-based culture media (data not shown).

CFU/mL counts were determined after the direct contact of the bacterial *E. faecalis* suspension with CTZ and Ultrapex® pastes. No *in vitro* studies were found evaluating the antimicrobial activity of Ultrapex® paste using CFU/mL counts; however, its clinical use has resulted in successful outcomes in eliminating signs and symptoms in patients.[19]

The present study reinforces the notion that the antimicrobial performance of endodontic pastes depends not only on their chemical composition but also on their formulation quality, handling characteristics, and interaction with the substrate. Our findings showed clear discrepancies between laboratory-standardized CTZ preparations and the commercially acquired compounded CTZ paste (CTZ UNAM), which is consistent with the variability reported in previous studies evaluating antibiotic-based formulations.[15,16,18]

This study confirmed the *in vitro* activity of the two antimicrobial agents which are part of the CTZ paste formulation, as well as the antimicrobial properties of zinc oxide and eugenol. CTZ paste is considered a compounding formula, meaning that there is no standardized control over the quality and source of its components, which may influence its effectiveness in inhibiting bacterial growth. This was evident since the CTZ (UNAM) formulation showed no antibacterial activity, even when the antibiotic content was used at or above the minimum inhibitory concentration (MIC) values described by CLSI. Bacterial growth was comparable to the untreated control group, with uncountable colony numbers, indicating that the acquired paste may not have had the expected composition or concentration of active antibiotics. This observation suggests the need for chemical analysis of the CTZ paste used in this study to verify its formulation and content.[9]

It is important to emphasize that the objective of the present study was to evaluate the *in vitro* antimicrobial effectiveness of commercially available and laboratory-prepared pastes against reference strain of *Enterococcus faecalis* rather than to assess their chemical composition. The CTZ(UNAM) paste was used as acquired, reflecting is acrual clinical use. Therefore, although chemical characterization could provide additional information regarding formulation variability, it was beyon the scope of this study and does not compromise the interpretation of the antimicrobial results obtained.

Ultrapex® demonstrated a moderate but statistically significant reduction in *E. faecalis* counts, reducing bacterial load by approximately two logarithmic units. Although this effect was limited compared with CTZ (LAB), it aligns with the known mechanism of calcium hydroxide–iodoform pastes, which exert antimicrobial effects through hydroxyl ion release and sustained alkaline pH.[19] However, *in vitro* models have shown limited efficacy of calcium hydroxide pastes against facultative anaerobes such as *E. faecalis*, explaining the incomplete inhibition observed in this study.

The performance of CTZ pastes differed markedly depending on the formulation. The CTZ (UNAM) paste—despite being widely used in clinical settings—did not reduce bacterial growth compared with the control. This result is supported by evidence indicating that compounded CTZ pastes can present substantial variability in antibiotic concentration, stability, and even degradation when stored under non-controlled conditions.[18,19] The oily phase separation observed suggests inadequate homogeneity, which likely hindered the diffusion and direct contact of active compounds with bacterial cells. Similar inconsistencies have been described by Silva et al. (2019), who emphasized that CTZ variability significantly affects reproducibility in laboratory studies and possibly in clinical outcomes.

In contrast, the CTZ (LAB) formulation—standardized from raw antibiotics and freshly prepared—showed the greatest antimicrobial potency, including complete inhibition in the paste form. This finding is consistent with the classical evidence of Sato et al. (1992) and Takushige et al. (2004), both of whom demonstrated that when CTZ is precisely mixed in correct proportions, it produces strong antibacterial activity against both anaerobic and facultative microorganisms commonly present in necrotic primary teeth.

Our study further highlights the methodological importance of evaluating non-diffusible pastes using CFU/mL quantification rather than diffusion-based tests, as agar-based methods may falsely suggest lack of activity for materials with limited diffusion, such as Ultrapex®. The direct-contact approach employed in this study allowed a more accurate assessment of the actual antimicrobial effect of the pastes.

Clinically, the superior performance of CTZ (LAB) underscores the potential of CTZ when prepared under controlled, standardized conditions. This suggests that the inconsistent clinical outcomes reported for CTZ may be attributable to formulation variability rather than inherent limitations of the material. Standardization of CTZ preparation could therefore enhance its predictability and reliability in pediatric pulpectomy, particularly in cases where rapid bacterial reduction and minimal instrumentation are required. By contrast, the lack of antimicrobial effect observed in CTZ (UNAM) highlights the need for regulatory oversight of compounded dental materials to ensure therapeutic efficacy and patient safety.

Overall, the differences observed in this study emphasize that not all CTZ pastes behave similarly, and that formulation quality plays a decisive role in antimicrobial performance. Further studies using chemical characterization techniques (HPLC, mass spectrometry) are warranted to determine the stability and concentration of antibiotic components in commercial CTZ pastes.

CONCLUSION

Although CTZ paste was proposed over 50 years ago as an alternative pulpal treatment for primary teeth, in vitro studies evaluating its ability to inhibit bacteria associated with pulpal infections are limited. In the present study, Ultrapex® paste demonstrated significantly greater inhibition of bacterial growth than the control group. However, CTZ (LAB) paste, which was prepared in a laboratory, demonstrated greater antimicrobial efficacy against the *Enterococcus faecalis* strain. This strain is considered to be clinically relevant in cases of pulpal infection. These results suggest that CTZ paste has clinical potential as an effective therapeutic alternative, offering shorter application times and favourable outcomes, provided the correct concentrations of each component are present in the paste. Adding eugenol to the CTZ mixture improved its antimicrobial activity, providing further evidence of its efficacy against *E. faecalis*.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Muscle Atrophy Secondary to Spinal Cord Injury: A Global Understanding

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ABSTRACT

Introduction: Spinal cord injuries are widely acknowledged for their profound impact on quality of life. Consequently, there is a particular interest in comprehending the molecular and genetic pathophysiological mechanisms that are distinctly associated with the development of muscle complications, such as atrophy. These complications are crucial features of spinal cord injury, leading to further mobility limitations and systemic disturbances in cellular homeostasis. **Objectives:** The present review aims to elucidate the molecular and inflammatory mechanisms involved in muscle atrophy that contribute to the deterioration of individuals with spinal cord injury. Furthermore, the current therapeutic options for this condition will be explored in this review, with the aim of providing a comprehensive approach to muscle atrophy in spinal cord injury and identifying potential therapeutic targets. **Materials and Methods:** This narrative literature review was conducted through online research in PubMed, SciELO, and Web of Science between October 2023 and January 2024. A search was conducted using keywords such as "spinal cord injury," "muscle atrophy," "oxidative stress," and "skeletal muscle," with the use of Boolean operators to refine the search. A total of 48 studies were selected based on specific criteria. **Conclusions:** The process of muscle atrophy that occurs after a spinal cord injury is characterized by the disruption of motor signals, which in turn results in damage to the neuromuscular junction. Furthermore, this process gives rise to a calcium imbalance, as well as the activation of pathways that lead to cell death and protein breakdown. Inflammatory cytokines have been demonstrated to promote catabolism by inhibiting protein synthesis and inducing atrogenes such as MuRF1 and MAFbx. Calcium-dependent enzymes have been demonstrated to contribute to protein degradation, thereby driving progressive muscle loss. These findings underscore the significance of timely, multidisciplinary interventions —integrating pharmacological, rehabilitative, and molecular strategies— to preserve muscle function and enhance outcomes in individuals with SCI.

Key words: muscle atrophy; spinal cord injury; treatment.

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RESUMEN

Introducción: en el presente estudio se aborda una introducción al tema en cuestión. Las lesiones de la médula espinal se caracterizan por su repercusión en la calidad de vida de los pacientes, por lo tanto, resulta de particular interés comprender los mecanismos patológicos moleculares y genéticos fuertemente asociados al desarrollo de complicaciones musculares, tales como la atrofia. Estas complicaciones son características esenciales de las lesiones medulares, ocasionando restricciones adicionales en la movilidad y alteraciones sistémicas en el equilibrio celular**. Objetivos:** El propósito de la presente revisión es esclarecer los mecanismos moleculares e inflamatorios implicados en la atrofia muscular, contribuyendo así a la degeneración de las personas afectadas por lesiones de la médula espinal. En este estudio, se explorarán las opciones terapéuticas actuales con el objetivo de proporcionar un enfoque integral al estudio de la atrofia muscular en la lesión de la médula espinal y de identificar potenciales dianas terapéuticas. **Métodos y materiales empleados:** Este estudio constituye una revisión narrativa de la literatura, llevada a cabo mediante una exhaustiva investigación en línea en las bases de datos PubMed, SciELO y Web of Science, entre los meses de octubre de 2023 y enero de 2024. Se emplearon palabras clave como "lesión de la médula espinal", "atrofia muscular", "estrés oxidativo" y "músculo esquelético", utilizando operadores booleanos para refinar la búsqueda. El presente estudio aborda un total de 48 estudios seleccionados mediante criterios específicos. **Conclusiones:** La atrofia muscular posterior a la lesión medular se caracteriza por la interrupción de las señales motoras, lo que resulta en la afectación de las uniones neuromusculares, un desequilibrio en el calcio y la activación de vías de muerte celular y degradación de proteínas. El incremento de los factores inflamatorios estimula el catabolismo a través de la inhibición de la síntesis de proteínas y la activación de los genes reguladores de la degradación muscular, tales como MuRF1 y MAFbx. Las enzimas dependientes de calcio contribuyen a la degradación de las proteínas, lo que resulta en una pérdida progresiva de la masa muscular. Los hallazgos subrayan la relevancia de las intervenciones multidisciplinarias tempranas, que integran estrategias farmacológicas, de rehabilitación y moleculares, con el propósito de preservar la función muscular y optimizar los resultados en individuos con lesión medular.

Palabras clave: atrofia muscular; lesión medular; tratamiento.

INTRODUCTION

Spinal cord injury (SCI) is a devastating condition in which anatomical or electrophysiological disruption of the spinal cord interrupts the communication between the central nervous system and the peripheral nervous system.[1] This disruption leads to motor neuron dysfunction distal to the injury site, resulting in various physical impairments, with muscle atrophy being one of the most frequent.[2] Beyond the loss of neural input and output, inflammatory and signaling pathways play a significant role in long-term complications often associated with muscular and bone dysfunction, leading to a continuously worsening condition and, eventually, a downturn in the prognosis after SCI.

SCI etiology, according to Yanbo Liu *et al.,*[3] is mostly attributed to traumatic causes such as falls, violent acts, and transit-related injuries. The most prevalent and incidental level of damage is the cervical region, which is also related to the most significant disability. Given the trend of increasing global life expectancy over the last decades (reaching up to 73.5 years in 2019), the average age of individuals with SCI tends to increase. With a worldwide prevalence of around 20.6 million cases estimated in 2019 and very high medical management costs per individual, SCI is a matter of concern for global public health.[4]

SCI can be classified based on the injury mechanism and the chronological stages in which it presents. Regarding the first classification, non-traumatic SCI occurs when neuronal deterioration is mediated by immune-related, degenerative or infectious mechanisms. In contrast, traumatic SCI involves functional and physical interruption resulting from the external application of force conditioning, a complete or partial neuronal dysfunction distal to the injured segment.[5]

In this last case, the sudden application of force to the spinal cord not only damages the neuronal tissue, but also compromises blood vessels and adjacent structures such as bone, muscles and connective tissue, resulting in ischemia and the release of multiple proinflammatory mediators that will condition and perpetuate the dysfunction. In terms of chronological classification, the time from injury up to 48 hours is designated as the acute stage of SCI, between 48 hours and 14 days as subacute, 14 days to 6 months as intermediate, and after 6 months of clinical progression, it is considered a chronic lesion.[5] Throughout this process, depending on its stage, individuals with SCI experience

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varying degrees of inflammation and activation of signaling pathways. The inflammatory process, which is ongoing, has consequences for both damaged tissue and the potential for new long-term complications related to musculoskeletal dysfunction. These include the loss of muscle mass and, eventually, muscle atrophy. The chronological stages of SCI facilitate a pathophysiological understanding of immunological mechanisms and emphasize the significance of time-dependent medical therapies that prevent individuals from progressing to muscular complications.

In view of the considerable clinical implications of spinal cord injury, a thorough review of the mechanisms of muscle atrophy is both appropriate and necessary. The topic has been identified as being of significant relevance, primarily due to three key factors. Firstly, there is the increasing global prevalence of the condition. Secondly, there is the ageing population. Thirdly, and finally, there is the impact of muscle atrophy on long-term functionality. The physical consequences of muscle atrophy are twofold. Firstly, it limits mobility and independence. Secondly, it contributes to secondary complications.

These include, but are not limited to, insulin resistance, osteoporosis and cardiovascular disease. The latter two have been shown to drastically worsen prognosis and increase healthcare costs. Despite the advances made in the field of medicine, this narrative review offers a structured synthesis of the existing evidence, highlighting the gaps in literature and identifying emerging therapeutic approaches tailored to the mechanism of muscle atrophy. The objective of this review is twofold: firstly, to integrate epidemiological data, molecular insights and current treatment strategies; and secondly, to support evidence-based care and guide future research.

METHODOLOGY AND MATERIALS

This is a narrative literature review performed through online research in PubMed, SciELO, and Web of Science between October 2023 and January 2024. Keywords such as "spinal cord injury," "muscle atrophy," "oxidative stress," and "skeletal muscle" were used. Boolean operators were used in the following combinations: "spinal cord injury AND muscle atrophy", "spinal cord injury" AND "muscle atrophy" AND "oxidative stress" AND "molecular mechanisms", IF ("spinal cord injury" AND "muscle atrophy") THEN "oxidative stress". The selection criteria included English and Spanish language publications, evidence-based, especially focusing on muscle atrophy after SCI. Publications in languages other than English or Spanish were excluded. Articles that did not meet the eligibility criteria were excluded and classified, with reasons including insufficient data, non-indexed articles, and studies published in languages other than English or Spanish. Selected articles were published in journals related to immunology, oxidative stress, neuroscience, neurorehabilitation, molecular science, and neurology, between 2000 and 2024. Articles focused on prognosis, psychological impact, economic involvement, future aiming, and ethics were also excluded from this literature review.

Results of the search: A total of 294 records were initially identified. 150 from PubMed, 20 from SciELO, and 124 from Web of Science. After removing 84 duplicates, 210 records remained. A screening review of 210 documents was conducted by examining titles and abstracts, leading to the exclusion of 120 articles. Twenty of the remaining documents were not retrieved. The 70 remaining articles underwent a full text evaluation to determine if they met the inclusion criteria or not. Finally, 48 studies were selected (Figure 1). No automation tools were used in the process.

RESULTS

Muscle atrophy

Skeletal muscle is a key component of physiological metabolic homeostasis, including energy metabolism, thermogenesis, as well as mechanical and protective functions. Since 40% of our total body weight is muscle, representing approximately 50% of the total protein body mass-it is expected that any pathological process might trigger consequences that not only affect muscular tissue but also impact multiple metabolic processes and molecular pathways.[6]

Muscular tissue is composed of excitable contractile myofibers, which can be classified into three types based on their oxidative rate: Type I (slow oxidative rate), also known as red muscle fibers; Type IIa (fast oxidative rate); and Type IIb (fast glycolytic rate), also called white muscle fibers, which are capable of hydrolyzing ATP faster than type I fibers.[6]

The term "muscle atrophy" is defined as a skeletal muscle disorder characterized by the progressive deterioration of functionality and muscle mass decline. The categorization of this condition may be facilitated by its underlying etiology, which can be defined as the causative agents that precipitate the onset of the disease. In this instance, etiology is

constituted by congenital diseases, which are characterized by the presence of protein mutations. These mutations are the primary causative agents of muscle atrophy. However, the present review highlights that acquired muscular dysfunction may also result from malnutrition, sepsis, or neural disconnection causing muscular atrophy. In such cases, the underlying condition and the person's immobilization create an imbalance between protein synthesis and degradation, triggered by multiple signaling pathways, such as the sphingosine 1-phosphate signaling axis activated by the release of TNFα in acute inflammation affecting skeletal muscle myotubes. The result of these events is the promotion of cellular apoptosis, muscle wasting, weakness, and muscle atrophy, thereby perpetuating muscular dysfunction and clinically leading to a decline in mobility.[6-9] Histologically, atrophied muscle is characterized by a significant reduction in myofiber diameters and a hypereosinophilic sarcoplasm. However, in denervation atrophy, as observed in SCI, the characteristic histologic feature includes myofibers that are compressed and have crowded nuclei, along with the.

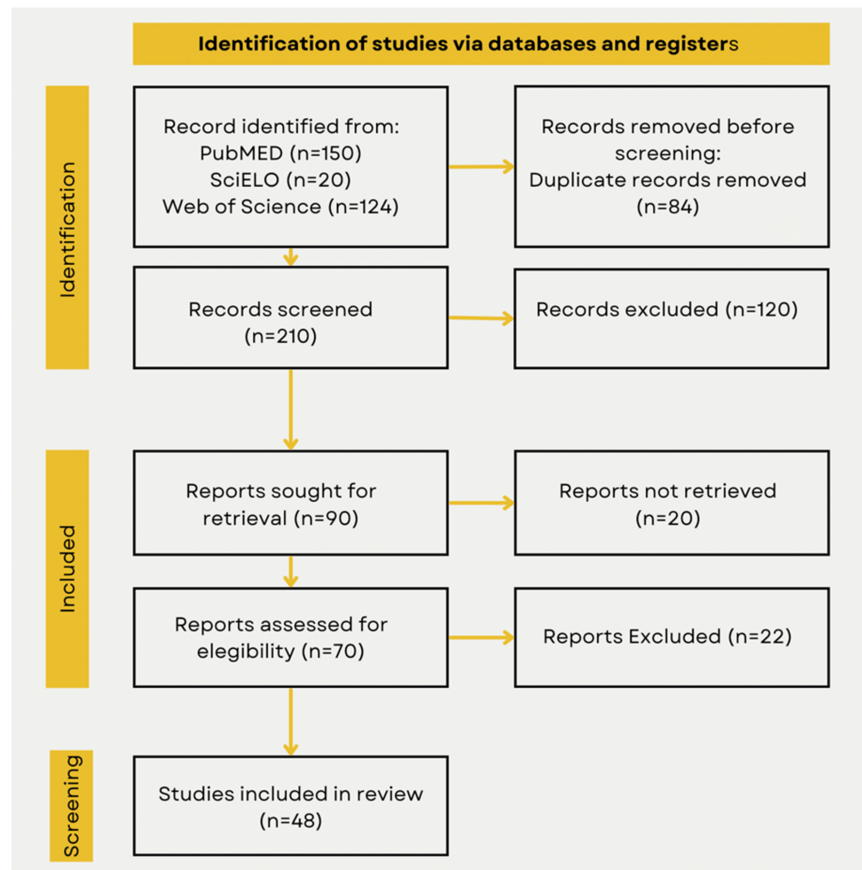

Figure 1. Prisma research flow diagram, 210 articles were screened, from PubMED, SciELO and Web of Science, after assessing eligibility, only 48 studies were included.

Loss of organelles and changes in muscular cell types. It is important to note that in animal models, a specific tendency to change the type of muscle fiber has been observed in type II fibers in cases of atrophy secondary to disuse, cachexia and malnutrition.[10]

After SCI, the disconnection of the neural network and the resulting lack of muscular movement lead to atrophy. In the complex neuronal relation between the spinal cord and skeletal muscle, there are three types of motor neurons that are crucial in the pathophysiology of muscular

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atrophy: Alpha, Beta and Gamma motor neurons. Since alpha motor neurons contain extrafusal fibers and are found predominantly at neuromuscular junctions, they act as force regulators. Gamma motor neurons have intrafusal fibers distributed largely in muscle spindles which act as somatosensorial mechanoreceptors, therefore modulating the length of muscular fibers during contraction. Finally, Beta motor neurons contain both extrafusal and intrafusal fibers.[7,8] Studies have shown that whenever there is a sudden neuronal interruption, motor neurons can become highly excited promoting deep hyperreflexia and even spasticity.[9] With the interruption of the synaptic communication between any given motor neuron and its corresponding neuromuscular junction, the motor endplate undergoes a degeneration process, resulting in the inability to eliminate acetylcholine, consequently leading to the accumulation of substrates such as calcium and the initiation of apoptotic processes in skeletal muscle.[9]

Consequently, the process of muscle atrophy after any SCI is not solely attributable to a sudden absence of neural input; it is also associated with various molecular pathways and molecular phenomena, including mitochondrial dysfunction, increased oxidative stress, inflammation, and the activation of multiple proteolytic enzymes that contribute to muscle wasting, cell apoptosis, and muscle atrophy.

Signaling Pathways, Inflammation and Proinflammatory Cytokines Involved in Muscle Atrophy

Muscle atrophy is a condition mediated by "atrogens", which are activated through various proteolytic pathways associated with this pathological process.[9] Given the complexity of the factors involved in these associated mechanisms, it is essential to understand some of the key elements intimately related to this common complication of SCI.

After a traumatic event involving the spinal cord, multiple factors and signaling pathways are activated to respond to the macro and microscopic damage caused by the initial insult. Although these elements initially aim to contain further damage and eliminate cellular debris, they eventually contribute to the perpetuation and development of muscle atrophy. Cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 1/6 (IL-1, IL-6), and tumor necrosis factor-like weak apoptosis inducer (TWEAK) act as pro-inflammatory mediators and are directly linked to muscle catabolism through the down-regulation of mRNA translation and blockage of muscle protein synthesis by inhibiting the PI3K/Akt pathway and promoting the transcription of atrophy-related genes (e.g. MuRF1, MAFbx).[11]

Following SCI, an increased intracellular calcium concentration has been identified as the starting point of a cascade of events that culminate in the participation of calcium-dependent proteases called calpains. These enzymes function as catalysts in proteolytic processes and contribute to the inhibition of various signaling pathways that ultimately result in muscle destruction.[11] The role of Calpain in calcium-mediated protein decomposition is to inhibit and catalyze the proteolytic events involved in muscle atrophy. Caspases are implicated in the loss of muscle fibers, as they play an essential role in apoptosis.[12] Additionally, the recruitment and activation rate of autophagic lysosomes that degrade protein-based material, is particularly important. These lysosomes show heightened activity during SCI and have preference for glycolytic type II muscle fibers in their autophagic process, as seen in Figure 2.[13]

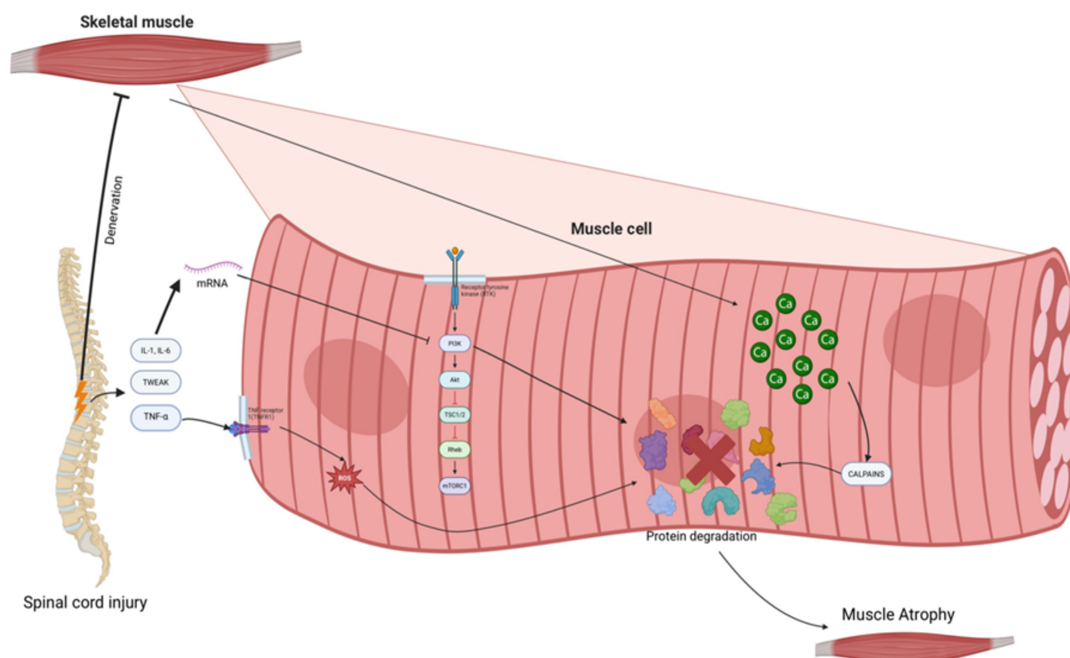

FIGURE 2. This image illustrates the complex network of molecular pathways leading to muscle atrophy following spinal cord injury (SCI). Key pro-inflammatory cytokines: TNF-α, IL-1/6, and TWEAK, initiate cascades that inhibit anabolic pathways such as PI3K/Akt and promote protein degradation via calpains, caspases, autophagy, and the ubiquitin-proteasome system. This intricate interplay underscores the multifactorial nature of SCI-induced muscle atrophy. Figure self-made in Biorender. IL: interleukin, mRNA: messenger ribonucleic acid, TWEAK: tumor necrosis factor-like weak inducer of apoptosis, TNF: tumor necrosis factor, PI3K: phosphatidylinositol 3-kinase, ROS: reactive oxygen species, mTORC1: mechanistic target of rapamycin complex 1, Ca: Calcium, Rheb: Ras homolog enriched in brain.

Another mechanism of muscle atrophy and catabolic induction is the ubiquitin-proteasome system, which consists of various enzymes and proteins that work together to degrade proteins, including ubiquitin itself, and a 26S proteasome responsible for degrading the polypeptides formed by the system, thereby promoting protein replacement.[6] Some ubiquitin-related proteins are of special interest because of their transcription factors, such as O-type forkhead transcription factor (FOXO), which plays an essential role in muscle atrophy by participating in many protein destruction pathways.[14]

The function of the Ubiquitin-E3 ligase muscle ring finger-1 (MuRf1) is of particular interest, as this gene has been found to be profoundly linked to muscle mass loss in recent studies and is recognized as a molecular marker of muscle atrophy. Another strongly involved ligase is muscle atrophy F-box/MAFbx (Atrogin-1), which has been observed to act as a mediator of myofibril degradation, making it a key component of these pathophysiological mechanisms.[15] It is worth mentioning that in many animal models, the expression of these factors can be significantly increased following SCI.[6,15,16]

Despite the existence of multiple catabolic mechanisms involved in SCI, we can also find factors that activate anabolic pathways, such as the signaling stream mediated by insulin-like growth factor-1 (IGF-1) that results in the activation of phosphatidylinositol-3 kinase (PI3K)/Akt, promoting hypertrophy and nerve regeneration.[9] Unfortunately, inflammation caused by SCI can significantly affect the concentration of substrates needed for these anabolic functions, thus impairing these capabilities and ultimately preventing muscle mass regeneration. Additionally, it has been observed that this down-regulation can persist chronically in subjects with a complete spinal cord section.[16]

As previously mentioned, there is a very complex interaction between molecules, genes and signaling pathways that lead to muscle mass reduction. Among them, TNF-α has multiple physiological functions related to immunity and inflammatory response. In addition, it is also known for

its involvement in apoptotic events when it is attached to the TNF receptor-1 (TNFR1). This results in the activation of multiple substrates that trigger the production and accumulation of reactive oxygen species, the transcription of proapoptotic protein mediators, and the activation and recruitment of caspases, which are closely linked to myofibrillar degradation and muscle mass loss.[17,18]

Additionally, individuals with pathological signaling pathway activation secondary to SCI present several mechanisms that promote muscle atrophy. Among these, myostatin, within the TGFβ superfamily, is of particular interest due to its expression being limited to skeletal muscle.[19] Myostatin regulates the transcription of genes such as MuRF1 and Atrogin1 through the phosphorylation of Smad complexes (Smad2/Smad3, and later Smad4), limiting muscle growth and playing a major role in muscle mass loss.[9,19] Equally important, there are multiple types of tissue and cell bodies capable of secreting interleukin-6 (IL-6), including adipocytes, cardiomyocytes, leucocytes and even skeletal muscle.[18] Specifically in skeletal muscle tissue, IL-6 plays an important role in the activation of muscle satellite cells. By doing so, it contributes to the development of new muscle cells; however, it has been observed that constant and long-term exposure to IL -6, in conjunction with other factors such as TNF-alpha, can promote muscle atrophy.[9,18]

This phenomenon also applies to TWEAK, belonging to the TNF superfamily, which is primarily produced in tissues undergoing active inflammation, such as the muscle and connective tissue involved in SCI. It is also linked to proteolysis and oxidative stress through nuclear factor kappa-light-chain-enhancer of B cells (NF-kappa-B).[9] In addition, fibroblast growth factor-inducible 14 (Fn14) acts as a TWEAK receptor, and the binding of these two components has been proven to induce tissue remodeling and fibrosis.[20] Thus, both TWEAK and TWEAK/Fn14 are involved in the pathological remodeling of skeletal muscle, leading to atrophy through their continuous activity in injured individuals.

Oxidative stress and Mitochondrial dysfunction

Mitochondrial biogenesis is regulated by various factors, including peroxisome proliferator-activated receptor-γ coactivator (PGC-1α), a transcriptional coactivator that is highly expressed in skeletal muscle. Through various signaling pathways, PGC-1α can increase the transcription of mitochondrial genes in response to certain stimuli, such as increased energy demand, as seen in SCI.[9,21] Moreover, PGC-1α is closely linked to the upregulation in antioxidant factors. Thus, PGC-1α provides oxidative stress protection not only through mitochondrial regulation and functions such as reactive oxygen species (ROS) detoxification and oxidative phosphorylation but also by promoting diverse antioxidant substrates that prevent oxidative damage and ensure mitochondrial survival.[21]

However, PGC-1α levels can significantly decrease with inflammation. Mitochondrial dysfunction invariably leads to an imbalance in the production and elimination of reactive oxygen species (ROS), which consequently promotes signaling and proapoptotic pathways in muscle cells.[9] It is known that due to mitochondrial function, ROS are inevitably produced, however, this production coexists in a delicate balance with their elimination through antioxidant agents. Therefore, any stimulus that aggravates this balance results in the accumulation of ROS and is reflected in the deterioration of cellular functions.[22]

Finally, in conditions with long-term inflammatory environments, such as SCI, the downregulation of PGC1-α might perpetuate and increase oxidative damage. In addition to the harmful mechanisms triggered by inflammation that can inevitably result in muscle atrophy, it is important to note that muscle atrophy itself is also related to an increase in oxidative stress, creating a self-sustaining inflammatory loop initiated by the initial injury and further perpetuated by muscle atrophy.[23] Exogenous antioxidant therapy (e.g. vitamin D) has been recommend recently to reduce or reverse oxidative stress and support the muscular rehabilitation of individuals after SCI.

Bone Implications in Muscle Atrophy

The lack of motion in the limbs following a SCI, which leads to the absence of mechanical stress, has a particularly negative impact on the physiological functions of the bone, since mechanical stress is one of the main promoters of bone remodeling.[24]

Moreover, the delicate balance between bone formation and resorption depends on multiple factors that stimulate complex signaling pathways, resulting in cell differentiation into either osteoclasts or osteoblasts. On one hand, osteoblasts can produce macrophage-colony stimulating factor (M-CSF) and RANKL, which, when combined with RANK, trigger a signaling cascade leading to the differentiation of osteoclasts. These osteoclasts, along with cathepsin k and hydrochloric acid, are responsible for bone resorption. On the other hand, the recruitment of

osteoblasts leads to the formation of new bone segments along with osteocytes.[25]

When limb motility is lost, the mechanical stimuli that the bone is designed to carry is no longer available. Therefore, in the absence of these mechanical stimuli, osteocytes will not produce signals that promote bone remodeling, leading to a decrease in bone tissue, osteoporosis, and a greater risk of fracture.[24,25] Interestingly, elevated IL-6 concentrations have been identified in individuals with SCI, attributed to osteoclast-like cell stimulation observed in bone marrow cultures of people with paraplegia.[25] Some studies suggest that the loss of bone quality may begin shortly after an SCI and can persist chronically if not adequately addressed.[24,26]

Systemic Implications of Muscle Atrophy

Beyond the outcomes that specifically concern the musculoskeletal system, there are multiple systemic repercussions that can substantially deteriorate the prognosis of the SCI individuals. The inability to perform certain activities due to SCI leads to a significantly sedentary lifestyle. Given the musculoskeletal conditions resulting from SCI, there is a tendency towards insulin resistance and alterations in carbohydrate metabolism, as well as difficulty in thermoregulation and accumulation of adipose tissue. These factors may lead to a higher incidence of cardiometabolic morbidities, atherosclerosis, dyslipidemias, chronic renal disease, among other conditions, compared to people without SCI.[27–29] There has also been a clear association between low muscle mass and cardiovascular events, specifically in SCI individuals with cervical or thoracic injuries that compromise the cardiorespiratory function. Physical rehabilitation to maintain muscle mass has been proven to improve pulmonary capacity and systemic cardiovascular function.[29]

Pharmacological management approach

To date, no pharmacological strategies have demonstrated significant reduction or reversal of muscle atrophy. Nonetheless, given the number of signaling pathways involved in the pathophysiology of this process, several therapeutic options are currently available, and some others are still under study, which could radically change the outcomes for individuals with SCI.

As previously mentioned, muscle atrophy in SCI involves the disruption of many molecular pathways. Due to the variety of these alterations, there are as many clinical approaches as there are disturbances. For instance, it has been identified that up to 60% of men who have suffered an SCI present with gonadal dysfunction and, as a result, a decrease in total testosterone levels.[15]

Testosterone, an androgen with anabolic effect, is strongly linked to the maintenance of proper musculoskeletal function. It not only contributes to increasing muscle mass but has also been observed to promote protein synthesis by activating PI3K/Akt pathways, thereby decreasing the expression of MAFbx, FOXO1, MuRF1, and other genes involved in muscle atrophy.[15,16,30,31]

Additionally, testosterone is involved in neuronal recovery processes and is known to have neuroprotective characteristics against oxidative stress.[32] However, it is important to consider the risks involved in testosterone hormone therapy, with one of the main concerns being prostate growth in males.[14] As a result, this therapy is not yet a viable option to prevent muscle wasting and atrophy on its own, as it requires further studies.

It is evident that mitochondrial dysfunction, in conjunction with the subsequent accumulation of ROS, constitutes a pivotal factor in the progressive decline in muscle function and mass. Consequently, a therapeutic approach that has been proposed is the utilization of antioxidants. It has been hypothesized that the utilization of vitamins, including but not limited to vitamin E and vitamin D, may result in a reduction in the prevalence of muscle atrophy. It has been determined that the incorporation of polyphenols could play a pivotal role in the prevention of mitochondrial dysfunction. This process involves the elimination of the accumulation of ROS and the reduction of the pro-inflammatory stimulus that promotes and perpetuates skeletal muscle damage.[33]

Various other options have been explored as part of the pharmacological approach to muscle atrophy, targeting the signaling pathways involved in this condition. For instance, pharmacological agents that inhibit myostatin, which, as previously discussed, is an important factor restricting muscle growth through the regulation of genes linked to muscle atrophy, although they have not been proven to be completely effective in SCI management. It has been hypothesized that the potential exists for the utilization of β2-agonists to promote protein synthesis and activate PI3K/Akt signaling pathways, thereby achieving anabolic benefits.[14,19,34] However, despite their individually promising therapeutic effects, these approaches are not sufficient to completely meet the needs of an individual with muscle atrophy secondary to SCI, as there is currently

no pharmacological option available that can address the complex signaling network involved in this condition.[16]

Genetic, cellular and other managements in development and study

The possibility of a genetic approach to the management of muscular atrophy has significantly developed over the last few decades. However, current limitations in this field still represent a hurdle for its implementation as a definitive treatment.

The potential exists for the application of both muscle-derived and non-muscle-derived stem cells to mitigate this complication. In addition, endeavors to identify efficacious therapies for the management of muscle atrophy have resulted in the exploration of strategies such as the controlled use of tetanus neurotoxin. These have the capacity to be transported by the axon after their internalization at the neuromuscular junction, thereby activating a series of complex mechanisms that lead to the disinhibition of the involved motor neurons.[35,36] However, despite being excellent therapeutic alternatives, they still have technical complications that prevent their full therapeutic implementation.[33,35]

Functional Electrical Stimulation

Functional Electrical Stimulation (FES) involves the application of controlled electrical impulses to neuromuscular junctions and muscle fibers via surface electrodes to induce muscular contraction. It is widely used as a rehabilitation and injury prevention technique. When combined with resistance training, this electrical stimulation can be a highly effective therapeutic resource, resulting in muscular hypertrophy and improving overall bone and muscle health.[37,38] There are several modalities for this electrostimulation therapy, including FES-cycling, FES-rowing, FES-assisted, and electro stimulated resistance training (NMEST-RT).[38]

Maintaining proper muscle function through FES has been shown to increase muscle mass, improve blood circulation, and enhance cardiorespiratory performance. It is also associated with a reduction in complications related to muscle atrophy, such as fractures, venous thrombosis, and glucose intolerance.[39,40] Furthermore, evidence suggests that FES can be combined with other therapies, such as blood flow restriction, to achieve positive effects and long-term results.[41]

The integration of assistive technologies with therapeutic approaches has proven to be highly beneficial for individuals' functionality. Despite partial or complete denervation secondary to an SCI, upper motor stimulus may still be produced. In recent years, work has focused on developing neuromechanical prosthetic models, designed to replicate or enhance the mechanical or neurological function of extremities affected by the lack of neural input, acting as exoskeleton that promote functional recovery.[42,43]

It is worth mentioning that even with the growing development of devices featuring neuromechanical technology, any instrument that facilitates the performance of activities for the individuals is considered an assistive device, whether it is a wheelchair or a robotic device. Despite the availability of multiple assistive devices, physical rehabilitation and muscular resistance exercises are essential components of the comprehensive management of SCI individuals, as they have proven to be especially effective in preserving muscle mass and preventing complications.[44]

Physical Rehabilitation

The fundamental principle of physical rehabilitation lies in the systematic repetition of movements, with or without resistance, aimed at improving physical capacity and muscle strength.[45] Evidence supports the necessity of physical therapy in individuals with muscle atrophy secondary to spinal cord dysfunction, regardless of whether it is of traumatic origin.[46] However, an interdisciplinary approach is crucial for creating a personalized program that adjusts the required number of sessions, intensity, and exercises to the specific needs of everyone, according to the severity of their condition. Thus, extremely encouraging results can be obtained, which, when combined with the previously mentioned therapeutic efforts, can drastically change both the prognosis and the quality of life of people with spinal cord injury.[45,47,48]

CONCLUSION

Spinal cord injury (SCI) induces profound skeletal muscle atrophy through mechanisms that extend beyond the loss of neural stimulation. Current evidence demonstrates that muscle wasting is driven by a complex interaction of chronic inflammation, mitochondrial dysfunction, oxidative stress, and proteolytic systems such as the ubiquitin-proteasome pathway, calpains, and caspases. Proinflammatory cytokines,

including TNF-α, IL-6, and TWEAK, not only inhibit anabolic signaling pathways like PI3K/Akt but also promote the expression of key atrogenes such as MuRF1 and Atrogin-1, directly contributing to protein degradation. These changes affect type II muscle fibers and are compounded by elevated intracellular calcium levels, which exacerbate apoptotic and autophagic activity. Although anabolic pathways mediated by IGF-1 attempt to counteract muscle loss, their effects are limited by the persistent inflammatory environment. Furthermore, the downregulation of PGC-1α disrupts mitochondrial biogenesis and antioxidant defense, intensifying oxidative damage. Collectively, these findings highlight the multifactorial nature of muscle atrophy in SCI and establish the need for targeted therapeutic strategies that address the underlying molecular mechanisms to preserve muscle mass and improve systemic outcomes in affected individuals. Clinical implications of the present study urge physicians to implement multidisciplinary clinical approach; clinicians must consider not only musculoskeletal rehabilitation but also systemic monitoring and intervention to mitigate secondary muscle atrophy complications. Early personalized interventions integrating pharmacological, rehabilitative, and assistive technology to optimize patient outcomes and preserve long-term functionality. Future research should focus on the identification of molecular targets and biomarkers to guide tailored therapies.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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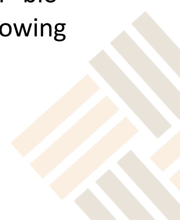

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