

II Workshop Internacional em Biotecnologia na Saúde:

Caminhos da Inovação

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DE 08 A 10 DE ABRIL DE 2025

II Workshop Internacional em Biotecnologia na Saúde: Caminhos da Inovação

De 08 a 10 de abril de 2025

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Faculdade de Medicina de Botucatu

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM. DIVISÃO
TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CAMPUS DE BOTUCATU – UNESP
Bibliotecária Responsável: **Ana Clara Gatto – CRB 8/10577**

Workshop Internacional em Biotecnologia na Saúde (2 : 2025 : Botucatu)
Anais do II Workshop Internacional em Biotecnologia na Saúde : caminhos da inovação [recurso eletrônico], 8 a 10 de abril de 2025 / Comissão organizadora Marjorie de Assis Golim ... [et al.]. - Botucatu : Faculdade de Medicina, 2025
Ebook

Programa de Pós-Graduação em Pesquisa e Desenvolvimento (Biotecnologia Médica)
ISBN: 978-65-5067-082-5

1. Biotecnologia. 2. Tecnologia de bioprocessos. 3. Bioprodutos. 4. Biotecnologia (Tendências). I. Golim, Marjorie de Assis. II. Universidade Estadual Paulista (UNESP), Faculdade de Medicina, Botucatu.

CDD 660.6

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ANAIS DO EVENTO: II WORKSHOP INTERNACIONAL EM BIOTECNOLOGIA NA SAÚDE: CAMINHOS DA INOVAÇÃO

Tema: Biotecnologia

VIRUCIDAL ACTIVITY ANALYSIS AGAINST SARS-COV-2 OF ESSENTIAL OILS FROM PROTIUM HEPTAPHYLLUM, HEDYOSMUM BRASILIENSE AND BLEPHAROCALYX SALICIFOLIUS.

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Introduction: SARS-CoV-2, the causative agent of COVID-19, has triggered a global health crisis, driving the search for new therapeutic strategies. Among natural compounds with antiviral potential, essential oils (EOs) have gained attention due to their broad bioactive properties, including antimicrobial, anti-inflammatory, and antioxidant activities. In this context, plant species from Brazilian biodiversity, such as *Protium heptaphyllum* (PH), *Hedyosmum brasiliense* (HB), and *Blepharocalyx salicifolius* (BS), are known for their rich chemical composition, suggesting potential antiviral activity. Objectives: This study aimed to evaluate the virucidal activity of EOs from these species against SARS-CoV-2, exploring their potential as natural antiviral agents. Materials and methods: The cytotoxicity of the EOs was assessed in VERO (ATCC® CCL-81™) and BEAS-2B (ATCC® CRL-9609™) cells using the Neutral Red assay after 72 hours of exposure at 37°C and 5% CO₂. Additionally, the antiviral activity was tested against the Omicron XBB.1.5 subvariant, expanded in VERO cells, using a multiplicity of infection of 0.04. To better understand the potential antiviral effects, two experimental approaches were employed: (i) the cellular approach, in which EOs were applied simultaneously with the virus and cells to assess their potential block infection, and (ii) the virucidal approach, where EOs were pre-incubated with the virus before cell exposure to evaluate their ability to inactivate viral particles. Results and discussion: The cytotoxicity assay showed that HB and BS had low toxicity in BEAS-2B cells, allowing a working dose of 78.125 µg/mL. In VERO cells, PH and HB exhibited moderate toxicity, with a working dose of 19.5 µg/mL, while BS showed higher toxicity (IC₅₀ = 0.53 µg/mL, working dose = 0.25 µg/mL). Regarding antiviral activity, HB showed no effect, whereas BS and PH demonstrated virucidal activity at 0.075 µg/mL and 9.75 µg/mL, respectively, suggesting direct viral inactivation. Variations in toxicity and antiviral activity between VERO and BEAS-2B cells underscore the need for appropriate cellular models in therapeutic evaluations. The greater efficacy of the virucidal approach suggests that these EOs may act by directly inactivating viral particles before cellular infection, a mechanism shared by other natural antivirals. This highlights their potential not only for inhalation therapies designed to reduce viral load but also for surface disinfection applications. However, further studies are essential to assess their stability, safety, and effectiveness in more complex models. Conclusion: PH and BS essential oils showed virucidal activity against SARS-CoV-2 Omicron XBB.1.5 at non-toxic concentrations for BEAS-2B cells, suggesting a direct viral inactivation mechanism. They hold potential for inhalation therapies or disinfectants, but further studies are necessary.

Palavra(s) Protium Heptaphyllum; Hedyosmum brasiliense; Blepharocalyx
chave(s): Salicifolius; SARS-CoV-2; Vero CCL-81; BEAS-2B; Antiviral.

ANALYSIS OF THE TRANSCRIPTIONAL PROFILE OF RESPIRATORY SYNCYTIAL VIRUS (RSV) AT PEDIATRIC PATIENTS AND A549 CELLS

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Introduction: Respiratory syncytial virus (RSV) is a leading cause of severe respiratory illness in children under five, resulting in approximately 3.6 million hospitalizations worldwide. It is also a major contributor to infant mortality, responsible for one in 50 deaths in children under five and one in 28 deaths among infants aged one to six months. Despite its impact, the molecular mechanisms behind RSV severity remain poorly understood. **Objectives:** To compare the transcriptomic profiles of pediatric patients with RSV infection with the transcriptomic response of RSV-infected A549 lung epithelial cells. By comparing these models, we aimed to identify similarities and differences in the host response. **Materials and methods:** RNA-Seq libraries were prepared with Illumina Stranded Total RNA Prep and sequenced on the NextSeq 500 platform. Raw sequence data for A549 samples were retrieved from the NCBI SRA, and the GRCh38/hg38 human genome assembly was obtained from UCSC Genome Database. Data quality was ensured using Trim Galore v0.6.5, followed by read alignment with HISAT2 v2.2.1 and transcript assembly with StringTie v2.2.0. Differential gene expression analysis was conducted using DESeq2, and ShinyGO v0.82 was used as a track to enrichment analysis. **Results and Discussion:** We conducted a comparative transcriptomic analysis of pediatric patients with RSV infection and RSV-infected A549 lung epithelial cells. Among the 62 genes differentially expressed between mild and severe patient cases, 26 were also altered in the A549 model. Notably, CXCL5, SLFN13, MMP9, and ITGB3 were linked to viral infections and immune response, while immune-related genes such as SELP, ITGA2B, and SLFN12L were exclusive to patient samples, indicating a more complex immune regulation in vivo. Enrichment analysis of the shared genes identified 10 significant pathways ($p = 0.05$), including IL-17 and TNF signaling, actin cytoskeleton regulation, focal adhesion, and Rap1 signaling, all associated with viral pathogenesis and immune response. The partial overlap between patient and A549 transcriptomes highlights the strengths and limitations of epithelial models in RSV research. While A549 cells effectively capture epithelial antiviral mechanisms, they do not fully reflect the complexity of the host immune landscape. Future studies should focus on co-culture systems, lung organoids, or ex vivo models to better integrate immune components and deepen our understanding of the molecular mechanisms underlying RSV severity. **Conclusion:** This study revealed key molecular pathways linked to RSV severity, emphasizing the role of inflammatory signaling in disease progression. While the A549 model captured epithelial responses, its limitations in immune representation underscore the need for advanced models. Further research is crucial to strengthen associations between

gene expression and clinical outcomes, enhancing our understanding of RSV pathogenesis and guiding targeted therapies

Palavra(s) chave(s): Respiratory Syncytial Virus; RNA-Seq; Pediatric Patients and A549 Cells.

THE EFFECTS OF THE COMBINATION OF FOOD-DERIVED ISOTHIOCYANATES AND CURRENT IMMUNO/CHEMOTHERAPY ON HETEROTYPICAL COLORECTAL CANCER SPHEROIDS

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Colorectal carcinoma (CRC) has a poor prognosis, and the available treatment strategies, including chemotherapy combinations with 5-fluorouracil (5-FU) and irinotecan (CPT-11) or 5FU with the bevacizumab (BVZ) immunotherapy, do not yield the desired outcomes. Therefore, the research for alternative drug combinations is highly encouraged, including the use of food derived from bioactive compounds, including isothiocyanates. Although these compounds have shown preventive effects in epidemiological studies and in vitro/in vivo bioassays, their full antitumor potential remains unexplored. Hence, we evaluated whether allyl (AITC) or phenethyl (PEITC) isothiocyanates enhance the antitumor effects of current chemo/immunotherapy regimens for CRC in an in vitro model. Heterotypic spheroids formed by HCT-116 CRC cell line and CCD-18Co colonic fibroblasts (ultra-low adherence plates, in 3:1 proportion) were treated with PEITC, AITC, BVZ, CPT-11 or 5-FU individually for half-maximal inhibitory concentration (IC50) calculation in cell viability (MTT) data (24 and 48h). Then, 1/5 and 1/10 of the IC50 of PEITC (8.8 and 4.4µM) and AITC (1513 and 757µM) were combined with the 5-FU+CPT-11 or 5-FU+BVZ doublets at 1/5 (1+30µM or 1+15µM) and 1/10 (0.5+15µM or 0.5+7.5µM) of the IC50 for each compound — similarly to plausible serum and tissue levels — and cell viability and the Combinatory Index (CI, 1= additive, <1 synergism, >1 antagonism) were also evaluated. Data were analyzed by one-way ANOVA and post hoc Tukey test (p<0.05). PEITC did not enhance the antitumoral effects of the doublet regimens (5-FU+CPT-11 or 5-FU+BVZ). Nonetheless, AITC promoted the BVZ+5-FU-induced reduction in spheroid viability, whilst it did not alter CPT 11+5-FU effects. Indeed, the CI indicated antagonism between the PEITC and the therapies (PEITC+5-FU+BVZ: 7.64; PEITC+5-FU+CPT-11: 1.06), while AITC had synergistic effects with 5-FU+BVZ doublet (AITC+5-FU+BVZ: 0.97; AITC+5-FU+CPT-11: 1.77). These data suggest that AITC promotes the antitumor effects of current immunotherapy plus chemotherapy regimen for CRC, and further mechanistic investigations are warranted.

Palavra(s) chave(s): Chemotherapy; Colorectal Carcinoma; Fibroblasts Immunotherapy; phenethyl isothiocyanate.

A METHODOLOGICAL COMPARISON FOR THE VALIDATION OF SEMI-QUANTITATIVE FLOW CYTOMETRY ASSAYS: THE MODEL APPLIED TO THE QUANTIFICATION OF PERIPHERAL BLOOD LYMPHOCYTE POPULATIONS

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INTRODUCTION: Flow cytometry is a technology widely used in clinical laboratories for diagnostic purposes, particularly in the study of immunological disorders. Immunophenotyping is a fundamental tool in this process, as it allows the simultaneous assessment of several cellular characteristics with precision and speed. However, it is imperative to implement rigorous methodologies to ensure the integrity of each analytical step, thereby maximising the accuracy of the results obtained by this method. Therefore, this study addresses the main practices related to internal and external quality control, their relevance, and their impact on each phase within the clinical laboratory. **OBJECTIVE:** In this context, this study aimed to develop and validate a protocol for semiquantitative flow cytometry assays using lymphocyte immunophenotyping as a model. **METHODOLOGY:** a comprehensive review of the literature on immunophenotyping, quality control and validation in flow cytometry was performed using the PubMed database and International Clinical Cytometry Society (ICCS) protocols. employed involved a comparative analysis of lymphocyte immunophenotyping protocols on two different flow cytometers (FACSCalibur®- BD and DxFlex®-Beckman Coulter). The evaluation encompassed parameters such as precision, accuracy, reproducibility, linearity and robustness of the new method. **RESULTS:** A Standard Operating Procedure (SOP) was formulated for the validation of semi-quantitative flow cytometry assays. This procedure was adapted to the requirements of the laboratory and incorporates acceptance criteria for each component of the validation. **CONCLUSION:** The validation of the new method guarantees the reliability and quality of the lymphocyte immunophenotyping results. Moreover, the SOP has facilitated the standardization of the validation process for semiquantitative tests for this specific application, as well as for other prospective applications pertinent to laboratories utilizing flow cytometry as a methodological framework. This enhancement of services and pursuit of exceptional standards is a testament to the efficacy of the Standard Operating Procedure.

Palavra(s) chave(s): Flow Cytometry; Immunophenotyping; Validation Studies; Lymphocytes; Quality Control.

ANALYSIS OF THE INCIDENCE OF POSITIVE CASES OF INFLUENZA A AND B IN PATIENTS TREATED IN THE MUNICIPALITY OF BOTUCATU FROM 2021 TO 2024

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Acute Respiratory Infections (ARIs) represent a significant global public health challenge due to their high transmissibility and impact on vulnerable populations. Among the main etiological agents, the Influenza virus stands out for its ability to cause seasonal epidemics and pandemics. This study aimed to assess the incidence of Influenza A and B in patients from Botucatu between 2021 and 2024, analyzing two distinct groups: patients treated at the Clinical Hospital of the Botucatu Medical School and participants in the CeVIVAS project. The study investigated the prevalence of viral subtypes, correlated epidemiological data of clinical relevance, and evaluated the effectiveness of diagnostic methods. The results revealed a predominance of Influenza A across all analyzed years. In the first group, the positivity rate was 97.46%, with subtype A H3 being the most prevalent in 2022, while subtype A H1N1 dominated in 2023 and 2024. In the second group, 87% of samples tested positive for Influenza A, with H1N1 being the most frequent subtype. In both groups, incidence peaked during autumn and winter, aligning with global trends. Demographic analysis indicated higher susceptibility among children and the elderly, reinforcing the need for targeted preventive strategies. The distribution of cases across Basic Health Units showed that Cohab 1 had the highest number of positive cases, accounting for 20.37% of the total. The comparison between diagnostic methods demonstrated a high efficiency of the rapid test as a screening tool, although RT-PCR remains essential for diagnostic confirmation, ensuring greater accuracy in case detection. These findings highlight the importance of continuous epidemiological surveillance to support more effective public health policies for Influenza control.

Palavra(s) chave(s): Epidemiology; Human Influenza; Seasons; Diagnostic Tests.

PHENOTYPIC ASPECTS OF THE CELLULAR IMMUNE RESPONSE TO VACCINATION AGAINST COVID-19 WITH THE CHADOX1 NCOV 19 IMMUNIZER

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Introduction: In the pandemic of SARS-CoV-2, the main strategy to protect the population was to develop a safe, effective and feasible vaccine that would induce neutralizing antibodies and prevent worsening of the infection. However, beyond the recognized impact on humoral immunity, vaccines also induce alterations in cellular immunity. This dual mechanism is widely considered to be an integral aspect of an ideal vaccine. **Objective:** The present study aims to assess the phenotypic characteristics of the cellular immune response to vaccination against SARS-CoV-2, using the ChAdOx1 NCOV-19 immunizer. **Methods:** Peripheral blood samples from 46 men unvaccinated/naive to SARS-CoV-2 were analyzed by flow cytometry to quantify subpopulations of lymphocytes (total, LTCD4+, LTCD8+), NK cells (total, NKdim/bright) and monocytes (total, classical, non-classical) at three time points: prevaccination (PV), after the first (1D) and second (2D) vaccine dose. The data were then subjected to statistical analysis using the Wilcoxon signed rank test using GraphPadPrism® v.6 software. **Results:** A significant increase in total lymphocytes ($p=0.0022$) and LTCD8+ ($p=0.0096$) was observed after 1D compared to PV. For T lymphocytes, in addition to an increase after 1D,

higher levels were also observed after 2D (PVx1D=0.0106; 1Dx2D: p=0.0283). A similar increase was observed in total natural killer (NK) cells after 1D compared to PV (p=0.0296). However, these levels decreased after 2D (p=0.0318), aligning with the PV trend. No statistically significant differences were observed in the CD4/CD8 ratio, LTCD4+ levels (cells/mm³ and %), and NK and monocyte subpopulations (%). The findings are consistent with the established correlation between specific CD4+/CD8+ T cell responses and milder stages of the disease, suggesting a protective role for these cells in viral infection and emphasizing the significance of NK cells in these conditions. Additionally, the suggestion has been made that the cellular memory immune response may be longer lasting than the humoral response. Conclusion: the results of the study indicate an increase in TCD8+ and NK cells following the administration of the first vaccine dose, suggesting the presence of an antiviral protective factor resulting from the initial stimulus.

**Palavra(s)
chave(s):** Vaccine; Cellular immunity; SARS-CoV-2.

DOSE-DEPENDENT EFFECTS OF APIS MELLIFERA VENOM IN AN ANIMAL MODEL AND A HISTOPATHOLOGICAL STUDY

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Bee stings are among the three leading causes of human envenomation. The *Apis mellifera* is considered a hybrid species with the highest lethality. A single bee sting injects, on average, between 140 and 150 micrograms of venom, and the median lethal dose (LD₅₀) of bee venom for humans varies based on body weight. Thus, a non-allergic individual weighing between 60 and 70 kg has approximately a 50% chance of death when subjected to 1,000 to 1,500 stings, highlighting the severity and potency of bee venom. This study focuses on inducing envenomation by *Apis mellifera* in an animal model to obtain the LD₅₀ and to observe the histopathological effects of this envenomation. Six mouse experimental groups were established based on subcutaneous venom administration at four distinct sites. Blood samples were subsequently collected via cardiac puncture to obtain plasma, while the brain, heart, lungs, duodenum, right kidney, and liver were collected for histopathological analysis. The organs were quickly placed into cryogenic tubes and frozen in liquid nitrogen. As result, the determination of LD₅₀ was counted through the number of animals that did not survive 48 hours after inoculation of increasing doses of venom. The result found in the test in mice presented an estimated LD₅₀ = 7.6 µg/g. Histopathological analysis of mouse tissues revealed that increasing venom doses induced progressively severe changes in the examined organs. At a dose of 3 µg/g, alterations were primarily restricted to the heart, showing early necrosis and initial signs of ischemic injury, with no significant changes in the kidneys or lungs. When the dose was increased to 30 µg/g, more pronounced effects were observed, including extensive cardiac necrosis, ischemic changes, acute tubular necrosis in the kidneys, and parenchymal retention in the lungs. At 100 µg/g, the damage was even more severe, with additional cardiac infarctions, more pronounced acute tubular necrosis in the kidneys, and significant pulmonary changes, such as increased thickening and aeration. These findings indicate a dose-dependent effect of the venom, with progressively greater organ damage at higher doses. This descriptive study may aid in the development of methods for more efficient pre-hospital care following bee sting incidents. Furthermore, the histopathological damage observed

suggests that affected individuals may experience long-term sequelae months after envenomation.

Palavra(s) chave(s): LD50; Apis mellifera; Histopathological Analysis; Toxicity.

EVALUATING THE SOFT SKILLS OF STUDENTS ENTERING HIGHER TECHNOLOGY COURSES

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Industry 4.0, characterized by the integration of advanced technologies, has caused transformations in labor relations. Organizations require workers with specific skills to meet the demands of technological development. Higher education courses need to adapt their curricula, because the lack of qualified professionals is an obstacle in the large-scale adoption of these technologies. Achcaoucaou et al. (2014) suggest that identifying student competence levels helps higher education course organizers to improve academic content and learning, since an academic experience is only considered productive when the level of competence reached at the end of the course is higher than the initial level. The objective of this project is to evaluate the soft skills of the students of the courses of Technology in Logistics and Industrial Production, through annual tests and mapping of soft skills. To make the project viable, a partnership was established between the Paula Souza Center, FATEC Botucatu and UNESP Botucatu. After the approval of the project at CEPE, applied research was started, quantitative and exploratory, to evaluate the six skills most required by industry 4.0: risk acceptance, creativity, teamwork, resilience, leadership and interpersonal relationships. The undergraduates were submitted to behavioral evaluation tests - BET, applied in the discipline of Entrepreneurial Education at the entrance of the course and subsequently the TAC were reapplied annually, through a computer program developed for this purpose. The evaluation of the responses was carried out by means of a five-point Likert Scale. The BET made between 2023 and 2025, showed that the classes had a good level and development of Soft Skills. As stated by Alberton and Beuren (2013), the interaction with new teaching methodologies allied to the professional environment favors the development of soft skills that are highly valued. The competencies in which students obtained the best results were Teamwork, Resilience and Leadership, in the evaluations of 2024 and 2025, 70% presented progression or maintenance of the level in comparison to the evaluation of 2023. The skills with the lowest progression index were Risk Acceptance, Creativity and Interpersonal Relationships, the latter being especially worrying, since 50% of the students showed regression in their development. According to Coda (2023), soft skills are not innate and can be developed, and their improvement requires continuous commitment to personal and professional growth. The results strongly suggest that the insertion of the discipline of Entrepreneurial Education, at the beginning of the Graduation stimulates the development of soft skills and allied to the committed individual with his inner change can increase the inclusion of these professionals in the labor market.

Palavra(s) chave(s): Behavioral skills; Entrepreneurship; Higher education; Industrial production; Industry 4.0; Logistics.

**CASE REPORT: DERMATOPATHY ASSOCIATED WITH
STAPHYLOCOCCUS SP AND ENTEROBACTER SP IN BOA
CONSTRUCTOR CONSTRUCTOR**

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Background: Boa constrictor is an aglyphous snake of the Boidae family. The maintenance of these specimens in captivity can be challenging due to the environmental factors, which can contribute to microorganism proliferation and the occurrence of opportunistic infections. Objective: The aim of this study was to describe the presentation of a dermatopathy in the species and the etiological agents involved. Methods: Thorough description of the clinical findings, diagnosis and treatment. Results: An adult Boa constrictor specimen maintained at a public visitation serpentarium in Botucatu, Brazil, presented multiple skin lesions, featuring skin thickening, discoloration, dysecdysis and a fragility that led to lacerations upon the slightest touching. Swab samples were obtained from a microbiological culture and an antibiogram. The analyses revealed the presence of Staphylococcus sp and Enterobacter sp. Based on the antibiogram results, intramuscular administration of enrofloxacin (10mg/kg) was introduced every forty-eight hours. Treatment also included bathing in 0.05% povidone-iodine solution, fluid therapy with Ringer's lactate solution (10ml/kg), and a single subcutaneous dose of vitamin C (100mg/kg). The animal is still being treated and monitored closely, showing a slight improvement in its symptoms. Discussion: Staphylococci are not often identified as a cause of pathologies in reptiles. However, their resistance to several drugs is a public health concern. Enterobacter spp. can also be important due to their ability to develop antibiotic resistance as well as their opportunistic behavior in susceptible organisms. Although the use of antimicrobial therapy is important for the management of bacterial infections, drug selection can be complex because of the lack of studies on the efficacy of antimicrobials in reptiles and their pharmacokinetics. Appropriate treatment should also consider the severity of the clinical condition. In addition to the antibiotics, topical agents such as chlorhexidine or povidone iodine solution can be used. It is also essential to focus on correcting possible risk factors - adjusting temperature and humidity conditions, cleaning the environment and substrates. In this study, treatment also included vitamin C, which can be used to stimulate the animal's immune system. Also, ascorbic acid deficiency could be associated with skin and oral cavity lesions in snakes, having already been linked to skin thickening and spontaneous skin ruptures in anorexic pythons and boas. It is known that skin lacerations can increase the risk of infection by opportunistic agents. Conclusion: This study revealed the presence of Staphylococcus sp and Enterobacter sp in dermatological lesions in a Boa constrictor constrictor. These etiological agents have also been described as part of the natural microbiome of snakes and may be associated with opportunistic infections in susceptible animals.

Palavra(s) Bacterial Infections; Boidae; Infectious Skin Diseases; Opportunistic
chave(s): Infections; Snake.

PREVENTIVE EFFECT OF ORA PRO-NOBIS (PERESKIA ACULEATA MILLER) ON THE HEPATIC OXIDATIVE STRESS OF RATS SUBMITTED TO AN ACUTE DOSE OF DOXORUBICIN

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Introduction: Cancer is one of the leading causes of death worldwide. Among the available treatments, doxorubicin (DOX) is an effective and widely used drug, but its side effects, such as oxidative stress (OS) are toxic to several organs, including the liver, which plays a major role in metabolism. *Pereskia aculeata* Miller, also known as Ora-pro-nobis (OPN), is a plant with antioxidant properties. This study aimed to evaluate the antioxidant effect of OPN extract on DOX-induced OS in the liver. Methodology: Wistar Rats were randomly separated into two groups: Control vehicle (C, n=20) and OPN (OPN= 20), receiving 125mg/kg of OPN by gavage for seven days (CEUA: 1429/2023). After, the animals were redistributed into four groups (n=10): Control (CTRL, intraperitoneal injection (IP) with NaCl 0.9%), DOX (IP of 4mg/Kg of DOX), CTRL+OPN (IP of NaCl 0.9%), and DOX+OPN (IP of 4mg/Kg of DOX). After 48 hours, the animals were anesthetized and euthanized. Hepatic OS markers were analyzed: lipid peroxidation by malondialdehyde (MDA) levels (nmol/g protein), protein carbonylation (CBO) (nmol/g protein) and oxidation (AOPP) ($\mu\text{mol/U}$ Choramin/mg protein). Comparisons were performed using Two-way ANOVA with Tukey's post hoc (parametric data) or Kruskal-Wallis test with Dunn's post-hoc test (non-parametric data). Data are presented as mean $\pm$ standard deviation or median (interquartile range); p values <0.05 were considered statistically significant. Results: The DOX group exhibited significantly increased OS markers: MDA (CTRL: 43.7(55.6-36.3) vs DOX: 126.6(153.5-99.2), $p<0.001$), CBO (CTRL: 4.8 $\pm$ 2.4 vs DOX: 9.4 $\pm$ 3.4, $p<0.001$), and AOPP (CTRL: 16.3(18.8-12.1) vs DOX: 35.7(39.1-24.0), $p<0.001$). Pretreatment with OPN significantly reduced these markers: MDA (DOX: 126.6(153.5-99.2) vs DOX+OPN: 88.3(100.5-84.9), $p=0.002$), CBO (DOX: 9.4 $\pm$ 3.4 vs DOX+OPN: 6.3 $\pm$ 1.1, $p=0.005$) and AOPP (DOX: 35.7(39.1-24.0) vs DOX+OPN: 21.5(24.9-18.2), $p=0.006$) levels. Discussion: DOX is known to cause liver damage by increasing OS, mainly through ROS generation and impairment of the antioxidant defense system. The liver plays a key role in metabolizing DOX, leading to its accumulation and exacerbating oxidative damage. Our results confirm that DOX acute administration significantly elevates hepatic OS markers, supporting its hepatotoxic potential. The protective effects of OPN observed in this study align with findings on other bioactive compounds, which have shown antioxidant properties in reducing DOX-induced OS in various organs. These results are relevant, as the dose used showed protection against OS in the liver. The fact that the extract is being offered daily and contains many antioxidants may have participated in the fight against ROS. This study provides new insights into the hepatoprotective effects of OPN. Conclusion: Pretreatment with OPN prevented increased hepatic OS markers of rats submitted to an acute dose of DOX, indicating a protective effect against DOX-induced liver damage.

Palavra(s) chave(s): Antioxidant; Functional Food; Redox.

RADIOLOGICAL SAFETY ASSESSMENT IN SENTINEL LYMPH NODE LOCALIZATION IN BREAST CANCER

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Introduction: The sentinel lymph node localization (SLNL) conducted through radioguided surgery occurs in the surgical center, which is considered a radiation-free area, and the professionals performing this surgery are regarded as members of the general public. Therefore, radiation exposures must be within the limits permitted for individuals of the public. Objective: To estimate the effective dose in SLNL procedures for professionals involved in the surgery of breast cancer patients. Methods: This prospective, cross-sectional study involved 55 patients undergoing SLNL from December 2023 to August 2024. Dose rate and exposure measurements were taken 0.3 m from the injection site and from the sentinel lymph node. From these data, the activity and dose rates in the breast and lymph nodes at the start of surgery were estimated from physical decay calculations, and the effective dose for the professionals involved in surgery was determined. Results: The average injected activity was 86.82 ± 36.52 MBq, with an average interval of 25.08 ± 2.63 hours from injection to the start of surgery. The effective dose at distances of 0.3m and 0.5m was 100.35 and 60.21 μ Sv, respectively. Therefore, the annual effective dose, in the most conservative scenario, at these distances of 0.3m and 0.5m was 311.21 and 112.03 μ Sv, respectively. Discussion: This study evaluated the same professionals involved in the procedure. It found that at distances of 0.3 m and 0.5 m, assuming constant positioning and maximum annual procedures, the effective doses were 405.86 μ Sv and 243.52 μ Sv, respectively. These values are about one-third lower than the 1,000 μ Sv annual public exposure limit set by the ICRP. Conclusions: This study showed that SLNL conducted at our institution results in safe exposure levels for the surgical team.

Palavra(s) Radioprotection; Breast Cancer; Sentinel Lymph Node; Sentinel Lymph
chave(s): node Localization.

ARE ANTI-THYROID ANTIBODIES INFLUENCING RADIOIODINE THERAPY IN GRAVES' DISEASE?

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Introduction: Graves' Disease (GD) is an autoimmune disease, considered the most common cause of hyperthyroidism/thyrototoxicosis. GD is characterized by lymphocytic infiltration of the thyroid gland and activation of the immune system. The autoantibodies present are: antiperoxidase antibody (anti-TPO), anti-thyroglobulin, and mainly

antibodies directed against the TSH receptor (TRAb). Radioiodine therapy (RIT) with iodine-131 (I131) is considered a low-cost and effective method for the treatment and clinical management of hyperthyroidism in GD. Objective: Given the importance of RIT for the treatment of GD, we aim to evaluate the influence of autoantibodies on the efficacy of RIT in patients with Graves' Disease. Materials and Methods: This is a cross-sectional, descriptive observational study, with retrospective collection of clinical, demographic, laboratory data, imaging exams, and follow-up after one year of RIT in patients with GD treated from 2013 to 2021. Results: A total of 171 patients with Graves' Disease treated with RIT and followed one year after therapy were evaluated. There were 129 (75.44%) females and 42 (24.56%) males, with a medium age of 45.29 ± 14.74 years, ranging from 12 to 86 years. Forty (23.39%) patients were asymptomatic and 131 (76.61%) presented symptoms of hyperthyroidism, with only 15 (8.77%) not using antithyroid medication. TRAb antibodies were evaluated in only 74 patients, being positive in 57 (77.03%) and 17 (22.97%) negative. Anti-TPO was evaluated in 151 patients, being positive in 114 (75.50%) and 37 (21.64%) negative. The mean administered activity of I131 was 20.36 ± 9.18 mCi, ranging from 5 to 50 mCi. The evaluation after one year of RIT revealed 160 (93.57%) patients with hypothyroidism/euthyroidism and 11 (6.43%) with persistent hyperthyroidism. The association between RIT results and anti-TPO presented $p < 0.0022$, whereas there was no significance between RIT results and TRAb. Discussion: The findings regarding the distribution of patients by sex are consistent with the literature, which shows a significantly higher prevalence of DG in women. Studies suggest that high levels of anti-TPO may be associated with a higher incidence of hypothyroidism after RIT, possibly due to the immunological influence of this antibody. Conclusion: The results showed that the evaluation of the outcome of radioiodine therapy with the anti-TPO antibody presented a significant level, revealing that the presence of this antibody is associated with the success of RIT. However, no significant level was obtained for the TRAb antibody, suggesting that the presence or absence of this antibody is not associated with the success or failure of radioisotopic therapy.

Palavra(s) chave(s): Graves' Disease; Radioiodine Therapy; Anti-TPO; TRAb; Iodine I-131.

EVALUATION OF THE SEROLOGICAL FOLLOW-UP OF PATIENTS WITH PARACOCCIDIOIDOMYCOSIS

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Paracoccidioidomycosis (PCM) is a granulomatous systemic disease endemic to Latin America caused by fungi of the *Paracoccidioides* spp. complex. Measurement of serum levels of anti-*P. brasiliensis* antibodies by double agar immunodiffusion (DID) is used as a control for disease cure. The rate at which antibody levels decline in PCM is unknown. The aim of this study was to analyze the decrease in anti-*P. brasiliensis* antibody titers during antifungal treatment of patients with PCM. A non-comparative (retrospective) cohort study was performed on patients diagnosed with PCM, who had detectable antibody titers at admission and achieved serologic cure with treatment, seen at the Department of Infectious Diseases from the Clinical Hospital of the Botucatu Medical School - UNESP, between 2008 and 2018. All data used in the study were obtained from medical records. The chi-squared or Fisher's exact test was used to compare categorical variables, and the Mann-Whitney U test for

continuous variables, with an α error of less than 0.05 considered significant. Forty-nine patients were included in the study. All were treated with cotrimoxazole (CMX) and achieved clinical cure, which was faster in those with low serum anti-*P. brasiliensis* antibody levels — pure up to 1:4 (142.5 days [111.0 - 232.0]) and moderate — 1:8 up to 1:32 (126.0 [89.0 - 182.0]) than in those with high levels - 1:64 or more (223.5 [173.0 - 319.0], $p=0.006$). The same was observed for serologic cure (low = 307.5 days [121.0 - 549.0], medium = 322.0 [238.0 - 762.0], high = 646.5 [520.5 - 1,183.0]; $p=0.009$). All patients with low baseline antibody levels achieved significant reductions (greater than or equal to the median) at three and six months. Patients with moderate and high initial antibody levels showed a one fold decrease at three months and a four fold decrease at six months. An inverse relationship was observed between the decrease in serum levels and the presence of altered erythrocyte sedimentation rate and gamma globulins at three and six months, respectively. The results of this study allow us to identify a pattern of decreasing antibodies in patients with PCM treated with CMX.

Palavra(s) chave(s): Paracoccidiodomycosis; Double Agar; Immunodiffusion Treatment.

EVALUATION OF EFFECTIVENESS ON THE USAGE OF PAPAIN TO ERADICATE THE BIOFILM OF BOVINE MASTITIS CAUSING STAPHYLOCOCCUS AUREUS 22, 29 AND 36

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Introduction: most bacteria in nature are found living in self-produced extracellular structures called biofilms. The matrix composition depends on the bacteria involved in its production but, in general, it is composed of extracellular polysaccharides (EPS), protein and DNA. These structures provide great improvement in bacteria survival. Biofilm forming bacteria are often related to persistent infections on humans and animals. The matrix provides resistance to treatment such as antibiotics, causing difficulties to treat it properly. The treatment requires administration of high doses of antibiotics, which causes adverse effects on patients and low efficiency on treating the disease. The presence of biofilms on health has a significant impact on survival chances and cost of treatment, reaching USD 4.3 million dollars around the world. This matter requires an innovative approach to reach better efficiency in animal disease treatments, reducing side effects and costs. The use of enzymes in cell therapy has gained attention in the last years and its substrate specificity reduces undesirable interactions with the body. In this research, papain (Sigma-Aldrich, St. Louis, EUA. Batch: 129K7005), a proteolytic commercial enzyme that can be extracted from *Carica papaya*, was administered on three bovine mastitis causing *Staphylococcus aureus* isolates, aiming to destabilize their biofilm matrix by decomposing the proteins present in it. Objectives: evaluate the efficiency of papain in eradicating the biofilm produced by 22, 29 and 36 *Staphylococcus aureus* isolates. Methods: Pre-inocula of each bacterium were prepared using 5 mL of brain-heart infusion broth supplemented with 0.5% glucose and diluted to reach 0.1 optical density. 96 well plates were used to distribute the broth and were taken to 37°C incubator for 24 hours and then washed once with sterile PBS. 6 different concentration conditions (1; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/mL) were prepared using papain,

McIlvaine buffer (40mM) and Milli-Q water. The conditions were distributed in the plates and a blank was left with no enzyme treatments for 4 hours at 37°C incubator. The plates received another PBS wash 0.5% (w/v) crystal violet were used to color the plates for 5 minutes. After that, the plates were washed 3 times with distilled water. The content left on the plates were resuspended with 30% (v/v) acetic acid for 5 minutes and then taken to a spectrophotometer at 595 nm for reading. Results: papain was capable of eradicating the biofilm of all 3 isolates. It eradicated 97 - 98% of the isolate 22, 94 - 96% of the isolate 29 and 77 - 98% of the isolate 36. Discussion: papain has presented great effectiveness in eradicating *Staphylococcus aureus* isolates biofilms, which demonstrate the importance of protein content in their biofilm matrix. Conclusion: usage of enzymes in biofilm related infections present great potential and may help future treatments with satisfactory research and development.

Palavra(s) chave(s): Bacteria; Biofilm; Enzyme Treatment.

COAGULATION DISORDERS AND HEMOSTATIC ALTERATIONS IN DUCHENNE MUSCULAR DYSTROPHY

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Introduction: Duchenne Muscular Dystrophy (DMD) is a severe neuromuscular disorder characterized by progressive skeletal and cardiac muscle degeneration, leading to premature mortality due to cardiorespiratory failure. While extensive research has focused on muscular degeneration, recent evidence suggests hemostatic dysfunctions, including an increased risk of thrombosis and bleeding events, in DMD patients. These abnormalities may be linked to chronic inflammation and endothelial dysfunction, which exacerbate disease progression. Objective: This study aims to investigate hemostatic alterations in a murine model of DMD by assessing specific coagulation parameters, including Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT), and D-Dimer levels, in dystrophic and control mice. Additionally, we evaluated potential sex-specific differences in coagulation parameters. Methods: Male and female mice from the C57BL/10ScCr/PasUnib (control) and C57BL/10-Dmdmdx/PasUnib (mdx) strains were analyzed at 30 days and six months of age. Blood samples were collected post-euthanasia from the caudal vena cava and processed for hemostatic assessments using an automated coagulometer. Histological analyses were conducted to evaluate muscle degeneration and inflammatory markers. Statistical comparisons were performed using Student's t-test ($p \leq 0.05$). Results: The dystrophic group exhibited significantly prolonged PT and elevated D-Dimer levels compared to controls, suggesting a hypercoagulable state. Additionally, dystrophic females presented a more pronounced increase in D-Dimer levels compared to their male counterparts, implying a possible hormonal influence. Histological analysis corroborated these findings, revealing greater muscle degeneration and inflammatory infiltration in mdx mice, particularly in the diaphragm. Discussion: These findings suggest that DMD is associated with hemostatic dysregulation, likely influenced by chronic inflammation and endothelial activation. The sex-specific differences observed in coagulation markers further suggest a role for hormonal regulation in hemostatic imbalance. Increased estrogen levels may contribute to a pro-thrombotic state in dystrophic females by enhancing coagulation factor synthesis and reducing anticoagulant activity. These findings highlight the need for further investigation into the mechanisms driving hemostatic dysfunction in DMD and its potential implications for disease management. Conclusion: This study provides evidence of significant hemostatic alterations in the mdx model of DMD, reinforcing the link between dystrophinopathy and coagulation dysfunction. Observed sex differences emphasize

the importance of considering hormonal factors in future research. Expanding this analysis to include aged dystrophic mice may help elucidate the progression of hemostatic disturbances over time, contributing to potential therapeutic strategies aimed at mitigating vascular complications in DMD patients.

Palavra(s) Duchenne Muscular Dystrophy; Mdx Mice; Hemostasis; Coagulation
chave(s): Disorders; Muscle Degeneration.

HIGHER SOLUBLE MICB LEVELS ARE ASSOCIATED WITH REDUCED NKG2D-EXPRESSING NK CELLS IN HEALTHY INDIVIDUALS CARRYING THE MICB*004 ALLELE

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The activating receptor NKG2D is present in all NK cell subpopulations and CD8+ T lymphocytes. It interacts with stress-induced molecules such as MICA, MICB, and ULBPs, which can be expressed on the cell surface under stress conditions. Upon NKG2D activation, cellular cytotoxicity is triggered. However, MICB can be cleaved by metalloproteinases, generating a soluble isoform (sMICB), which also interacts with the NKG2D receptor and can be internalized, leading to NK cell activity downregulation. This mechanism serves as an efficient immune escape strategy for tumor and virus-infected cells, influencing different pathological outcomes, and is a potential axis of immunotherapy. This study aimed to correlate plasma sMICB concentration with the total percentage of NK cells expressing the available NKG2D receptor in healthy individuals with known MICB alleles. To achieve this, peripheral blood NK cells were incubated with anti-CD3, anti-CD56, anti-CD16, and anti-NKG2D antibodies and analyzed by flow cytometry. Plasma sMICB quantification was performed using an enzyme-linked immunosorbent assay. Analyses were conducted based on allele type, categorizing individuals into three groups: MICB*002 RES, MICB*004 SUSC, and MICB*005 OTHER. Our results showed that the MICB*004 SUSC group exhibited a significant increase in sMICB levels compared to the MICB*002 RES group ($p = 0.0147$). Additionally, this group also displayed a significant decrease in the percentage of CD56bright NKG2D-positive subpopulations compared to the MICB*005 OTHER group ($p = 0.0253$). These findings suggest that the higher plasmatic sMICB levels observed in the MICB*004 SUSC group may contribute to the reduced availability of NKG2D receptors on NK cells, supporting previous hypotheses that associate this allele with increased severity of infectious diseases and tumors. Consequently, healthy individuals carrying the MICB*004 allele may be more vulnerable to these conditions. Therefore, identifying the MICB allele, particularly in individuals from highly admixed populations such as the Brazilian population, could help guide more targeted therapies to prevent disease progression.

Palavra(s) sMICB 1; Flow Cytometry 2; NKG2D Receptor 3.
chave(s):

USE OF HYALURONIC ACID FOR PAPILLA FILLING IN AESTHETIC AREAS

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Papillae with poor tissue volume in aesthetic areas adjacent to dental implants and / or teeth represent a major aesthetic concern and challenge for dentists in clinical practice. The treatment for these black spaces has been extensively studied in order to achieve satisfactory results in relation to these defects in the region of papillae. In order to obtain improvement in these results, the present study has as main objective, to evaluate the possible use of the hyaluronic acid gel (HA) aiming at reducing the elimination or reduction of vertical defects in the papillae in aesthetic areas. Sixteen patients of both sexes and of different ages, older than 18 years, whose inclusion criteria included the presence of deficient sites in aesthetic area (Black Space), over 18 years of age, obeying the criteria of inclusion and search exclusion. The study evaluated 19 poor sites. A total of 3 applications were performed, with intervals of 4 weeks between them. The selected patients signed a free informed consent form (EHIC), were guided and documented through photographs and initial intraoral scanning. The photographs were made using a photographic device for image standardization, developed and patented for the research, in which a professional camera of CANON T3i with macro lens (100mm) and circular flash was coupled. In addition to the photographic images, the Dentsply Sirona (Omnican) CAD CAM case scanning was also performed, in order to be able to later evaluate the two methods of measurement. The images obtained in the sequence were in the periods, initial (T0), immediately after the application (T1), after 1 month (T2), after 2 months (T3) and after 3 months (T4). Always following an image pattern obtained through the photographic device used in all patients in a standardized way, in order to obtain reproducibility of the images. A total of 3 applications were performed, with intervals of 4 weeks between them. A local topical anesthetic (XYLESTESIN SPRAY 10%) was administered and then injected from 0.1 to 0.2 mL of HA until the gel was extravasated in the deficient papilla, 3 mm above the tip, apically. Measurements of possible two — dimensional vertical gain in the papilla areas were performed using image analysis software (Image J) for the different periods (T0 - T4) and for the three — dimensional gains were made through the software of the scanner itself (Dentsply Sirona). The bi and three-dimensional data were statistically correlated with the type of defect that the patient presented initially and later with the different periods of evaluation. Thus, we conclude that the application of hyaluronic acid to papilla filling in the aesthetic area is a beneficial and minimally invasive treatment.

Palavra(s) chave(s): Hyaluronic Acid; Dental Papilla; Dermal Fillers; Gingival Retraction.

HEPATIC METABOLIC MODULATION BY CITRUS BERGAMIA LEAF EXTRACT (BLE) IN OBESE RATS

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Introduction: The growing prevalence of obesity presents concerning projections for 2030, with estimates of 29.6% for obesity. This disease is associated with metabolic alterations such as hypertriglyceridemia and hypercholesterolemia, which increase the risk of hepatic steatosis, a fat accumulation representing at least 5% of liver mass, characterized as Metabolic Dysfunction associated Fatty Liver Disease (MASLD). Evidence indicates that obesity significantly increases the risk of developing MASLD. The bergamot (*Citrus bergamia*) leaf extract (BLE), a source of bioactive compounds with hypolipidemic properties, stands out as a promising therapeutic agent in the management of dyslipidemias. **Objective:** Investigate the impact of BLE on hepatic metabolic profile in obese rats. **Methodology:** Wistar rats were distributed for obesity induction, receiving either a control diet (C) and high sugar-fat diet (HSF) for 20 weeks. After this period, animals were redistributed: C, C+BLE, HSF, and HSF+BLE, with BLE administered of 50 mg/kg/day by gavage for 10 weeks. Caloric intake (g/day), Final body weight (g) and adiposity index (AI,%) were measured. Plasma was used for biochemical analyses. In liver tissue, was performed Oil-red staining and measurements of cholesterol (mg/dl), CEUA: 1337/2020. Data is presented as means $\pm$ standard deviation or medians with interquartile range. The results were compared by two-way ANOVA with Tukey's post hoc test or Kruskal-Wallis with Dunn's post hoc test at a 5% significance level. **Results:** The HSF group demonstrated an increase in caloric intake ($p<0.001$), body weight ($p=0.003$), adiposity index ($p<0.001$), triglycerides plasma ($p<0.001$) and hepatic ($p<0.001$), cholesterol plasma ($p=0.028$) and hepatic ($p<0.001$), ALT levels ($p=0.003$), and a higher percentage of lipid droplets per area in liver slides stained with Oil Red ($p<0.001$) compared to the C group. BLE showed a reduction in body weight ($p<0.001$), adiposity index ($p=0.049$), and hepatic cholesterol levels ($p<0.001$), along with an increase in HDL cholesterol ($p=0.020$). **Discussion:** Regarding the improvement of lipid profiles by BLE, as increasing HDL and reducing hepatic cholesterol, Flavonoids in BLE, such as brutieridin and melitidine, along with derivatives like 3-hydroxy-3-methylglutaric acid, reduce total cholesterol by binding to the catalytic site of HMG-CoA reductase, inhibiting cholesterol synthesis and increasing HDL levels. **Conclusion:** BLE demonstrates promising therapeutic effects in alleviating hepatic obesity-related metabolic disturbances, improving lipid profiles.

Palavra(s) chave(s): Citrus Bergamia; Flavonoids; Antioxidants; Bioactive; Obesity; Phenolic Acids.

INVESTIGATION OF BIOMARKERS OF MERCURY EXPOSURE ASSOCIATED WITH FREQUENCY OF EXPOSURE IN RIVERSIDE POPULATIONS IN THE AMAZON REGION

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Mercury (Hg) contamination affects both human health and the environment. Highly toxic, bioaccumulative and biomagnifying throughout the food chain, it contaminates fish and all other beings along the food chain. Humans, as top predators of the food

chain, can suffer irreversible damage that mainly affects the nervous system. Hg can bind to proteins, inactivate them and even lead to death. The objective of this study was to develop/validate methodologies for the detection and evaluation of possible biomarkers associated with the frequency of exposure to Hg in plasma samples from the riverside population of the Jirau/Madeira River region in Rondônia. Samples from 28 individuals were collected using Noviplex™ cards based on the frequency of fish consumption by the population, verified through a qualitative dietary survey. The study was developed using a metalloproteomic approach using Graphite Furnace Atomic Absorption Spectrometry (GFAAS) for quantification of Hg, liquid chromatography-coupled mass spectrometry (Shotgun-LC-MS/MS) for identification of proteins and bioinformatics tools for studying their functions. The results of Hg determinations indicated a positive correlation between the concentration of total mercury (HgT) and the frequency of fish consumption. From this, the samples were classified into: G1, control group, lower concentrations of HgT and G2, G3 and G4, and groups with higher concentrations of HgT. After the proteolytic cleavage process, analyses of the abundance of proteins in the four groups of volunteers identified 132 proteins with positive and negative fold change and/or unique proteins, considering the comparison between groups G2, G3, G4 with G1. The results indicated positive fold change of the isoforms of Immunoglobulins (IGs), Albumin (ALB) and Ceruloplasmin (CP) in G2, G3 and G4, which may infer that the upregulation of these proteins are associated with the exposure of volunteers to Hg. The positive correlation of HgT concentrations with fish consumption by the groups of volunteers corroborates metalloproteomic data and suggests the isoforms of IGs, ALB and CP as candidates for biomarkers of Hg exposure in humans. It is noteworthy that Immunoglobulin kappa variable 3-20 (IGKV3-20) was expressed as a unique protein in G3 and G4, and CP was upregulated in G4, groups of volunteers that presented the highest concentrations of HgT. Since these proteins have coordination sites with soft base characteristics capable of interacting with Hg species, the data of the present study potentialize IGKV3-20 and CP as possible plasma biomarkers of Hg exposure. Thus, based on the results obtained, it can be inferred that the proteins/enzymes identified as downregulated, upregulated and/or unique proteins ($p < 0.05$) presented a positive correlation in the mercury bioaccumulation process depending on the frequency of fish ingestion and may act as potential biomarkers of Hg exposure in humans.

Palavra(s) chave(s): Biomagnification; Mercurial Species; Human Poisoning; Plasma Proteome.

ANTIOXIDANT STRATEGIES IN POULTRY HEALTH: PROTEOMIC INSIGHTS INTO PASSION FRUIT SEED AND PEQUI OILS FOR HEAT-STRESS CONDITIONS

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High temperatures in laying hen production induce physiological and hormonal changes, leading to increased oxidative stress and potential

macromolecular damage. To mitigate these effects, dietary adjustments, including antioxidant-rich vegetable oils, have been explored to improve bird performance under heat stress. This research evaluated the effects of supplementing layer diets with passion fruit seed oil (PFSO) and pequi oil (PO), in which may contribute to innovative nutritional strategies for sustainable poultry farming in challenging climates, while also exploring the impact on protein metabolism and expression, with proteomic analyses to better understand molecular adaptations related to heat stress resilience. Lohmann White hens (26 weeks old) were housed in cages within a climatic chamber under cyclic heat stress (31°C/32°C) for 8 hours per day. They were divided into three groups: 0.9% PFSO, 0.6% PO, and a control (CON). After an 84-day experiment, blood samples (one per unit, six per treatment) were collected and processed for plasma separation. Plasma was diluted with a 100 mmol L⁻¹ solution of urea, thiourea, and dithiothreitol (6 µg µL⁻¹ total protein). Then, 100 µL of diluted plasma underwent proteolytic cleavage with 80 µL of 0.05 µg µL⁻¹ trypsin. Peptide extracts were desalted via a C18 column, concentrated to 2 µL, and analyzed by LC-MS/MS (nanoACQUITY UPLC-Xevo QT MS, Waters, UK). Protein abundance variation was determined using at least two peptides with $p < 0.05$ (downregulated) or $p > 0.95$ (upregulated). The proteomics study revealed biochemical mechanisms by which PO and PFSO modulated adaptive responses in laying hens under heat stress, where the analysis identified upregulated proteins that connect supplementation with these oils to improved metabolic homeostasis, antioxidant defense and immunity, critical aspects for animal health. The protein relative abundance variance analysis identified the modulation of 96 proteins in the plasma proteome of laying hens exposed to cyclic heat stress. Supplementation with 0.9% PFSO upregulated Ovotransferrin, suggesting a role in iron homeostasis under heat stress. Increased Albumin may protect lipids from oxidation, while Apolipoproteins facilitate lipid transport for egg yolk formation. These changes enhance metabolic efficiency, immune function, and antioxidant defense, improving heat stress resilience. In the 0.6% PO group, Hemopexin and Ovotransferrin showed antioxidant and protective effects. The exclusive presence of Proteasome activator subunit 4 suggests adaptation to oxidative stress. Alpha-2-Macroglobulin and Serpina1 indicate a PO-modulated inflammatory response, while the vWFD domain-containing protein may support tissue integrity and cell adhesion under extreme heat. These findings highlight PO and PFSO as potential strategies for enhancing poultry health in challenging conditions.

Palavra(s) Laying hens; Vegetable Oils; Oxidative Stress; Shotgun LC-MS/MS;
chave(s): Plasma Blood.

MUCOADHESIVE GEL FORMULATED FROM SUSTAINABLE RAW MATERIALS: PHYTOCHEMICAL AND THERAPEUTIC EVALUATION OF AGAVE SISALANA AND PUNICA GRANATUM RESIDUES

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Oral cancer is a malignant neoplasm with a high incidence in regions such as Latin America and Southeast Asia, often associated with tobacco use and alcohol consumption. As an alternative to conventional treatments, topical formulations like mucoadhesive gels have been explored for their ability to deliver bioactive compounds directly to the lesion, providing localized anti-inflammatory, antioxidant, antineoplastic, and healing effects. Plant-based drug formulations have gained attention due to their lower incidence of adverse effects and the presence of secondary

metabolites such as saponins and phenolic compounds. Plants from the Agave and Punica genera offer sustainable sources of pharmacologically active raw materials. This study aimed to extract secondary metabolites compounds from Agave sisalana residues (SR) and Punica granatum peel (PR), by incorporating them into a mucoadhesive gel (600 µg/g of each extract), and evaluating its therapeutic potential. Phytochemical profiling was performed using Fourier-transform infrared spectroscopy (FTIR). Antineoplastic activity was assessed in vitro by the MTT assay in HSC-3 oral cancer cells at 24, 48, and 72 hours. Antioxidant activity was evaluated using DPPH and ABTS assays at concentrations ranging from 200 to 600 µg/g. Data were statistically analyzed using ANOVA followed by Tukey's test. FTIR analysis confirmed the presence of triterpenoid saponins, phenolic compounds, and polysaccharides in the formulation. Cell viability decreased to $70 \pm 0.05\%$, $50 \pm 0.05\%$, and $30 \pm 0.05\%$ at 24, 48, and 72 hours, respectively, with statistically significant differences compared to the control ($p < 0.05$). The ABTS assay showed inhibition rates of $63.62 \pm 0.05\%$ (200 µg/mL), $74.43 \pm 0.05\%$ (400 µg/mL), and $73.46 \pm 0.05\%$ (600 µg/mL). DPPH inhibition was $55.01 \pm 0.05\%$, $66.19 \pm 0.05\%$, and $67.10 \pm 0.05\%$ at the same concentrations. These findings demonstrate that the gel contains pharmacologically active secondary metabolites and exhibits significant antineoplastic and antioxidant activities. Therefore, the SR- and PR-based gel stands out as a promising candidate for the development of a topical phytopharmaceutical for use in dentistry, while promoting the sustainable valorization of agro-industrial residues widely discarded in Brazil.

Palavra(s) chave(s): Antioxidant; Saponins; Medicinal Plants; Mucoadhesive Gel; Oral Cancer.

BIOCOMPATIBILITY AND APPLICATION OF BAMBOO FIBERS IN DRESSINGS AND BANDAGES: A LITERATURE REVIEW

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Introduction: The use of natural fibers, such as bamboo, in the manufacturing of dressings and bandages is proving to be a promising strategy, particularly with the growing demand for sustainable solutions in biotechnology. Bamboo, an abundant and renewable raw material, exhibits characteristics such as flexibility, strength, and durability, making it a viable option for biomaterial production. Bamboo fibers have shown potential in wound treatment and infection prevention, proving effective in the development of dressings due to their properties that promote safe contact with biological tissues, minimizing the risk of adverse reactions and fostering quicker and more effective healing. Moreover, these fibers are biodegradable, low-cost, and widely available, making them a more sustainable alternative compared to synthetic materials, which often generate negative environmental impacts and high costs. **Objective:** To synthesize available knowledge on the use of bamboo fibers as a raw material for dressings and bandages, emphasizing their biocompatible, antimicrobial, and regenerative properties. **Method:** An integrative literature review was conducted, with analysis performed across the following databases: LILACS, PubMed, Scopus, Scielo, Cinahl/EBSCO, Embase, and Web of Science. Inclusion criteria considered studies from the last ten years in any language, full-text articles, experimental and clinical studies. Studies that were duplicates across databases, other literature reviews, animal studies, and inconclusive studies were excluded.

Results: A total of 931 publications were retrieved. After the exclusion of duplicates and irrelevant articles, 795 articles were analyzed based on inclusion and exclusion criteria, with 10 articles selected for the final review. Bamboo fibers exhibit biocompatibility, allowing for safe interaction with biological tissues, which reduces adverse reactions and promotes wound healing. These fibers also possess natural antimicrobial properties, which help prevent infections in open wounds, making dressings more effective in protecting against pathogenic microorganisms. Additionally, bamboo fibers demonstrated excellent moisture absorption capacity and high breathability, characteristics that promote tissue regeneration and create an ideal environment for healing. Discussion: Despite the observed benefits, the literature remains limited, particularly in relation to the clinical application of bamboo fibers in medical devices. The lack of in-depth clinical studies on large-scale use suggests the need for further research to validate the efficacy and safety of these materials. Conclusion: Bamboo fibers represent a viable alternative for the production of dressings and bandages, offering advantages such as sustainability and efficacy. This study not only mapped existing knowledge but also provided guidance for future investigations into the biomedical application of bamboo fibers.

Palavra(s) chave(s): Bambusa; therapeutics; bandages; biological dressings.

CROTALUS DURISSUS TERRIFICUS WITH YELLOW VENOM: A SURVEY OF SNAKES RECEIVED BY CEVAP BETWEEN 2016 AND 2024

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Background: Snake venoms are known for their biotechnological potential in research and prospecting for candidate molecules to create biopharmaceuticals. CEVAP is a research and biotechnology center with a serpentarium that is a reference for good management practices and venom extraction. The animals kept are made up of snakes delivered by the population or government agencies. The rattlesnake "Crotalus durissus terrificus" (Cdt) is the most frequently encountered venomous snake, and its venom contains the toxin of the main bioproduct of CEVAP, the heterologous fibrin biopolymer (HFB). It is known that Cdt venom has biochemical variability within the same subspecies and the enzyme L-amino acid oxidase (LAAO) influences the yellow color of the venom. The percentage of Cdt with yellow venom in the region can vary from 10 to 35%. The use of complete venom with all the toxins and yellow coloration is essential for the production of antivenom serums and HFB. Objective: The aim was to observe the quantity of Cdt with yellow venom among all those received by CEVAP in recent years. Methods: A retrospective survey of the Cdt received by CEVAP in the period from 2016 to 2024 was carried out. The arrival date and the color of the venom in the first extraction were observed on the entry records of each animal. Results: The recorded data showed that in 2016, 135 Cdt were received, 3 of which had yellow venom (2.2%); in 2017, 160 specimens were received with 4 yellow venom (2.5%); 2018 was the year with the highest number of receipts, with 200 animals in total and 7 of them with yellow venom (3.5%); in 2019, there were 183 Cdt, 8 of which had yellow venom (4.4%); in 2020, 99 Cdt with 3 of yellow venom (3%); in 2021, 46 animals were received,

this time none of which had yellow venom; in 2022, 61 Cdt were received, 4 of which had yellow venom (6.5%); in 2023, 86 animals arrived with 7 of yellow venom (8.1%); and in 2024, none of the 71 Cdt received contained yellow venom. Discussion: It is observed that from 2020 onwards there was a decline in the reception of animals, this being the year that the pandemic and lockdown process began. 2021 was the most critical year for the maintenance of the snakes, with only 46 new animals. From 2021 to 2024, the serpentarium had no significant number of newly arrived rattlesnakes again, even after the post-pandemic period. Conclusion: Studies on factors such as the participation of the population and government agencies in capturing and delivering venomous animals to CEVAP, as well as environmental impacts, are important to understand this significant drop in the number of animals received. Maintenance strategies to increase the lifespan and venom extraction of snakes in captivity, and to find ways to deal with the decrease in the stock of complete venom are important at this time since the low bioavailability of some molecules directly impacts the research and production of biopharmaceuticals on a large scale.

Palavra(s) chave(s): Crotalus Durissus Terrificus; Yellow Venom; Snakes; Received.

IDENTIFICATION OF MICRORNAS IN PATIENTS WHO DEVELOPED LONG COVID

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Long Covid (LC) is characterized by the persistence of symptoms of the disease for at least three months after infection with SARS-CoV-2, with no other identifiable cause. These symptoms can be multisystemic, with neurological, hemodynamic and respiratory disorders being the most common. Exacerbated activation of the innate immune response, mediated by cytokines, has been identified as one of the main factors in the pathogenesis of CL. MicroRNAs (miRs) play a fundamental role in the post-transcriptional regulation of gene expression and can directly influence the production of these cytokines. Therefore, the aim of this study was to identify microRNAs differently expressed in patients with CL. To this end, plasma samples from 20 individuals previously infected with SARS-CoV-2 were analyzed, 10 with persistent symptoms (CL) and 10 asymptomatic after infection (control group, CO). The analysis was carried out using nCounter® technology. The results revealed a total of 40 significantly differentiated microRNAs, of which 36 were up-regulated ($p < 0.05$; $r > 1.5$) and 4 down-regulated ($p < 0.05$; $r < -1.5$). The interactions between the microRNAs and the cytokines of interest (IL-17A, IL-6, IL-4, IL-2, IL-1 β , TNF- α , IL-10 and IL-12p70) were investigated using the TarBase and GeneMANIA databases. Altered miRs in LC suggest a relevant influence on the regulation of cytokines involved in the persistence of symptoms. These miRs are associated with inflammatory processes, cell proliferation, recruitment and activation of immune cells. Thus, our findings indicate that important miRs such as miR-3140-3p, miR-324-3p and miR-218-5p contribute directly to the pathophysiology of LC and lead to more effective approaches to diagnosing and treating the disease.

Palavra(s) chave(s): Long Covid; MicroRNA; Cytokine.

RADIOMETRIC SURVEY IN AREAS ADJACENT TO THE THERAPEUTIC ROOM

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Introduction: In Brazil, the incidence of several thyroid carcinoma (DTC) has increased over the years, and this malignancy has a high cure prognosis. Nuclear medicine is a medical field that uses unsealed radioactive sources to generate diagnostic images or targeted therapies, with Iodine-131 (I-131) being the most commonly used radionuclide for treating benign diseases and DTC. Thus, the American Thyroid Association recommends total thyroidectomy followed by radioiodine therapy (RIT) for DTC tumors larger than 4 cm. This therapy involves the oral administration of I-131, an unstable element that undergoes radioactive decay and emits beta particles with sufficient energy to induce apoptosis in residual surgical and neoplastic cells. Consequently, after ingesting I-131, the patient becomes a radiation source, requiring isolation in a therapy room (TR), which is shielded to prevent public exposure to radiation. **Objective:** To conduct radiometric measurements in areas adjacent to the RIT TR to verify whether radiation exposure is below the limits established by the National Nuclear Energy Commission (CNEN). **Materials and Methods:** Eight measurement points were established around the TR: six points with patients lying down, including P1 (internal door), P2 (external door), P3 (corridor), P4 (adjacent classroom), P5 (adjacent room balcony), and P6 (adjacent room), and two points with patients standing, represented by P7 (in front of the table) and P8 (under the TV). Dose rate measurements were taken after radionuclide administration and at patient discharge using a Geiger-Müller radiation detector. Subsequently, the annual dose for each point was calculated, as well as an estimate for the worst-case hospitalization scenario. **Results and Discussion:** The annual dose at all measurement points remained within the limits set by CNEN. However, in the worst-case hospitalization scenario, points P5, P7, and P8 exceeded the optimized limit for public exposure. **Conclusion:** Thus, a potential increase in hospitalization demand raises concerns about implementing measures to minimize radiation exposure in these areas.

Palavra(s) chave(s): Radiometric Survey 1; Iodine-131 2; Dose Rate 3; Therapy Room 4.

IS THERE A CORRELATION BETWEEN THE DAILY RADIOMETRIC SURVEY AND EXAM SCHEDULING?

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Introduction: Nuclear Medicine is a medical specialty that uses unsealed radioactive sources for diagnosis and therapy, which must be

administered to patients so they can be detected by the scintillation camera, temporarily turning them into radioactive sources as they circulate through the nuclear medicine facility. The radiometric survey assesses radiation levels in different areas of a nuclear medicine service. Objective: This study aims to understand how the facility's routine affects radiation levels in different areas. Materials and Methods: Data collection was conducted at 24 designated points for the radiometric survey, carried out from Monday to Friday in the morning and afternoon. Information on the number and types of examinations, the administered radioactive activity, and the radionuclide used was also collected. Statistical analysis was performed using The SAS System software. Results and Discussion: A total of 160 radiometric surveys were evaluated, identifying the patient rooms and restrooms, examination room, hot lab, and waste room as the areas with the highest exposure rates. The number of examinations and radioactive activity was higher on Mondays, Tuesdays, and Thursdays, respectively, with a predominance of procedures in the morning. The radiometric survey results by periods and days of the week also showed higher exposure levels in the aforementioned areas. Regarding the types of examinations, renal scintigraphy was predominant from Wednesday to Friday, myocardial perfusion scintigraphy on Mondays and Tuesdays, and bone scintigraphy on Mondays and Thursdays. The correlation between radioactive activity and different scintigraphy types showed a moderate correlation for bone scintigraphy and stress myocardial perfusion scintigraphy, and a strong correlation for resting myocardial perfusion scintigraphy. The correlation between radioactive activity and the number of examinations per period showed a strong correlation for both periods, demonstrating that a higher number of examinations results in higher circulating radioactive activity. However, when correlating the radiometric survey with the number, type of examinations, or radioactive activity, despite statistical significance, no strong correlation was found. This is likely because the "radioactive sources" were not confined to a single area but were instead circulated or distributed across different areas of the facility. Conclusion: This study presents great relevance and impact on the management of occupationally exposed individual (OEI), as it highlights the days of the week, periods, and locations of higher exposure rates, highest radioactive activities, and higher concentrations of exam scheduling, allowing the radiation protection supervisor a better assessment for the rotation and scheduling of OEI.

Palavra(s) Radiometric Survey 1; Area Monitoring 2; Examination Types 3;
chave(s): Radioactive Activity 4.

ANALYSIS OF THE INCIDENCE OF DENGUE-POSITIVE CASES AND SEROTYPE PREVALENCE IN THE MUNICIPALITY OF BOTUCATU IN THE YEAR 2024.

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Dengue is an arboviral disease of global importance, amplified by environmental and socioeconomic factors such as rapid urbanization, climate change, and inadequate sewage management. This study evaluated the geographic distribution, serotype prevalence, and the effectiveness of diagnostic methods used in the city of Botucatu in the

year 2024, utilizing data from the CeVIVAS project. The analysis revealed a predominance of the DENV-1 serotype (77.8%), while the other serotypes showed limited circulation. A higher concentration of cases was identified in specific urban areas, associated with high population density and a greater number of potential breeding sites. The diagnostic evaluation demonstrated high sensitivity of the rapid test (98.7%) for initial screening, but low specificity (19.5%), with RT-PCR reaffirmed as the most accurate method. The combination of methods, including NS1 antigen detection and IgM and IgG antibody testing, proved essential for greater diagnostic accuracy, particularly for reducing the immunological window and the occurrence of false negatives. The results reinforce the importance of integrated actions, such as strengthening epidemiological surveillance, educational campaigns to raise public awareness, and improvements in sanitary infrastructure. It was concluded that targeted strategies are fundamental for the sustainable control of dengue in the long term.

Palavra(s) Dengue Incidence; Diagnostic Tests; Serotype; Epidemiological
chave(s): Surveillance.

DEVELOPMENT OF SANDWICH ELISA FOR CROTALUS DURISSUS TERRIFICUS VENOM QUANTIFICATION IN BLOOD SERUM

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Snakebite accidents fall within the category of neglected diseases in tropical and subtropical countries. Brazil ranks third, with approximately 28,000 cases recorded annually, with the highest incidence occurring in rural populations and agricultural laborers. The *Crotalus* genus is particularly noteworthy in Brazil due to the occurrence of acute kidney failure (AKF) in clinical cases. In this regard, the World Health Organization (WHO) has declared the development of strategies to reduce 50% of snakebite accidents by 2030. The use of biotechnological techniques capable of quantifying venom concentration in the bloodstream before antivenom administration is crucial for determining the most accurate dosage for treatment. Enzyme-linked immunoassays (ELISA) have broad applicability in this context, allowing the quantification of circulating venom in the ng/mL range. Here we aimed to analyze the neutralization profile of F(ab)'₂ fragments derived from the purification of crotalic commercial monovalent antivenom (CCMA) and to develop a Sandwich ELISA test to determine venom concentration in patients envenomated by Brazilian rattlesnakes. The methodology of this project is under patent confidentiality. *Crotalus durissus terrificus* venom was obtained from the Center for the Study of Venoms and Venomous Animals (CEVAP) at São Paulo State University (UNESP), Botucatu, SP, under authorization from the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA). The CCMA, produced by the Butantan Institute, was provided through collaboration with the State University of Feira de Santana (UEFS). Blood serum from six patients envenomated by rattlesnakes, all over 18 years old, and from ten healthy patients was obtained from the Botucatu Clinical Hospital, with approval from the Ethics and Research Committee on Human Subjects of the University in association with the hospital. F(ab)'₂ fragments were obtained using a specific resin coupled to an affinity chromatography system. These fragments were also used to prepare a conjugate applied to the Sandwich ELISA assays. The calibration curve was constructed using the blood serum of healthy individuals and snake venom. The results showed that 0.125 µg of purified F(ab)'₂ fragments were enough to yield optical density values between 1.0-1.5 when tested with different

venom concentrations, similar to tests with 0.125 µg of CCMA. Based on the calibration curve with an R^2 of 0.98, venom concentrations of 5-10 ng/mL were detected in the serum of envenomated patients. The results of this project highlight the neutralizing potential of purified F(ab)'₂ fragments compared to CCMA and their high applicability for a Sandwich ELISA test capable of quantifying circulating venom in patients envenomated by Brazilian rattlesnakes.

Palavra(s) Snakebite; Antivenom; Crotalus; Venom Detection; Enzyme-linked
chave(s): Immunoassays; Blood serum.

METALLOPROTEOMIC STUDY OF MERCURY (HG) AND SELENIUM (SE) IN THE LIVER TISSUE OF PSECTROGASTER AMAZONICA (BRANQUINHA) AND RAPHIODON VULPINUS (CACHORRA) FROM THE MADEIRA RIVER, RONDÔNIA/BRAZIL

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Mercury (Hg) is classified as highly toxic and stands out among the substances posing a major threat to public health, according to the World Health Organization (WHO). In the Amazon, it has been observed that mercury concentration levels in the region exceed the global average, posing a severe risk to aquatic systems and, consequently, to local consumers. This study aims to understand the role of Selenium (Se) in counteracting the toxic effects of mercury through the identification of specific biomarkers. Liver tissue samples were collected and preserved in liquid nitrogen from eight individuals of the fish species *Psectrogaster amazonica* "branquinha" and eight individuals of the species *Raphiodon vulpinus* "cachorra", located in the influence area of the Jirau Hydroelectric Power Plant reservoir on the Madeira River, Rondônia. The determination of total mercury and selenium concentrations in the samples was performed using graphite furnace atomic absorption spectrometry (GFAAS). Hepatic proteome fractionation was achieved through 2D PAGE, and protein identification was carried out via mass spectrometry coupled with liquid chromatography (LC-MS/MS). Based on the mercury and selenium concentrations (CHg and CSe) determined by GFAAS, the molar ratios Hg:Se and Se:Hg were calculated, followed by the determination of Selenium Health Benefit Values (Se HBVs). The Se:Hg values were greater than one for both fish species, while the Se:HBVs were greater than five for *Psectrogaster amazonica* and greater than ten for *Raphiodon vulpinus*, thus indicating that both fish species have selenium reserves in their liver tissue necessary to control the toxic effects of Hg. The metalloproteomic data allowed the identification of 11 protein spots associated with Hg and Se in both fish species, with concentrations ranging from 9.70±0.14 to 28.44±0.31 mg kg⁻¹ and 16.15±0.21 of Hg and 43.12±0.51 mg kg⁻¹ of Se. LC-MS/MS analyses identified five metal binding proteins (MBPs) in the protein spots associated with Hg and Se in the liver proteome of *Psectrogaster amazonica* and eight of *Raphiodon vulpinus*, indicating the possible formation of Hg-Se complexes in the MBP structure, and that binding with Se could mitigate the toxic effects of Hg (Bataglioli, 2024). Analyses of the activities of the enzymes CAT, SOD and GPx, and LPO (Catalase, Superoxide Dismutase, Glutathione Peroxidase, and Lipid Peroxidation, respectively), demonstrated no occurrence of oxidative stress induced by Hg concentration, which corroborates the metalloprotein data.

Palavra(s) Aquatic Systems; Protein; Hepatic Proteome; Biomarkers; Metal Binding
chave(s): Proteins; Spots.

ARTIFICIAL INTELLIGENCE IN IDENTIFYING UNUSUAL CLINICAL CASES IN MENTAL HEALTH: AN INTEGRATIVE BIOTECHNOLOGY APPROACH

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Unusual cases in mental health, characterized by unique combinations of symptoms and specific intensities (mild, moderate, or severe), have emerged as important clinical phenomena, often disregarded by traditional approaches. These cases present specific and rarely identified sets of symptoms, making their understanding and intervention challenging. This study aimed to identify and analyze such unusual cases, seeking to understand their biopsychosocial characteristics and offer support for more effective and contextualized clinical interventions. To this end, an exploratory quantitative analysis was carried out based on structured clinical interviews with 624 individuals treated at specialized mental health units, located in several cities in the states of Paraná and São Paulo, between September and December 2024. The collected data were processed with imputation of missing values, normalization, and coding of categorical variables, ensuring quality and accuracy of subsequent analyses. The integrated use of statistical analysis techniques and AI, especially machine learning algorithms, allowed the identification of a significant group of unusual cases, corresponding to approximately 33.65% of the total initial sample of 624 participants (n=210). Unusual cases were defined by the exclusivity of the combinations between specific symptoms and reported intensities, not shared by other participants. A predominant mean age of approximately 16.6 years was observed, with a special emphasis on students, followed by retirees, teachers, housewives. Important biopsychosocial factors also stood out, such as frequent or constant social isolation, significant cognitive difficulties in daily decision-making and challenging, restrictive or highly protective family contexts. The application of artificial intelligence methods, especially unsupervised algorithms such as K-means and PCA, allowed the clear identification of three distinct clusters, each with specific clinical characteristics, indicating concrete paths for targeted and effective clinical interventions. The results point to the importance of concrete historical and social conditions in the development and maintenance of psychological symptoms, highlighting the relevance of socio-historical psychology as a fundamental theoretical framework for understanding these phenomena. The integrated and innovative use of artificial intelligence with traditional statistical techniques revealed complex clinical patterns that could not be easily identified by traditional approaches, highlighting the significant potential of this methodology in professional practice. This study, thus, opens concrete and promising possibilities for the use of artificial intelligence in clinical practice in mental health, strengthening the theoretical and practical understanding of contemporary psychological phenomena and suggesting paths for the humane and effective treatment of patients with complex and specific clinical profiles.

Palavra(s) Mental Health; Artificial Intelligence; Machine Learning; Clinical Patterns;
chave(s): Biopsychosocial Factors.

IN VITRO ANTIVIRAL ACTIVITY OF TERPINEOL AND β -CITRONELLOL AGAINST MPOX VIRUS

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Introduction: Mpox, a zoonotic infection, is categorized into Clade I, associated with higher mortality rates, and Clade II, linked to milder disease manifestations. Globally, since 2022, there have been over 117,000 confirmed cases and 260 deaths, primarily concentrated in African regions such as the Democratic Republic of Congo, Burundi, and Uganda. In Brazil, the first case of Clade Ib Mpox, with an estimated 10% lethality, was recently confirmed. Current antiviral treatments, such as Tecovirimat and Brincidofovir, lack robust human efficacy data. Terpenoids, known for their antiviral properties through interactions with viral proteins, offer a promising alternative. Compounds such as terpinen-4-ol and β -citronellol have demonstrated anti-influenza and anti-HIV activity via viral replication inhibition. Thus, these phytochemicals may provide an alternative to address the limitations of conventional Mpox therapies. **Objectives:** This study aimed to evaluate the antiviral activity of terpineol and β -citronellol against Mpox virus. **Materials and methods:** Cytotoxicity was assessed in VERO (ATCC® CCL-81™) cells using terpene concentrations from 10,000 to 0.3 $\mu\text{g/mL}$. MPXV was propagated and quantified by plaque assay. Antiviral efficacy was evaluated via pre-infection and during infection administration protocols. DNA was extracted from both the supernatant and cells of treated samples, followed by real-time PCR (qPCR). **Results:** Terpineol and β -citronellol exhibited cytotoxicity above 312.5 $\mu\text{g/mL}$ and 156.25 $\mu\text{g/mL}$, respectively. MPXV was quantified at 1.6×10^8 PFU/mL. Terpineol and β -citronellol reduced MPXV viral load across various concentrations in both direct virus incubation and cellular pre-treatment assays. **Discussion:** Terpineol and β -citronellol exhibited antiviral activity against MPXV in vitro, reducing viral load in both direct virus incubation and cellular pre-treatment assays. The absence of clear dose response suggests complex mechanisms. Although qPCR does not differentiate between infective and non-infective viral load, it offers high sensitivity and specificity, enabling the detection of viral genetic material at low concentrations. These results suggest both compounds have potential for MPXV antiviral research and therapeutic development. **Conclusion:** Terpineol and β -citronellol demonstrate promising in vitro antiviral activity against MPXV, suggesting potential therapeutic applications. Further studies are needed to elucidate mechanisms and assess in vitro and in vivo efficacy.

Palavra(s) chave(s): Terpene; Natural-products; Virology; Essential-oil.

DEVELOPMENT OF AN ANTIFUNGAL SOAP ENRICHED WITH PLANT EXTRACT FROM A CERRADO PLANT

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The increased preference for phytotherapeutic agents over synthetic compounds is attributed to their lower incidence of adverse effects and greater therapeutic efficacy. *Trichophyton rubrum*, the main etiological agent of cutaneous mycoses in Brazil, often displays resistance to conventional antifungals, which may cause adverse effects such as hepatotoxicity and hemolytic anemia. In this context, the Amaranthaceae family stands out for its production of diverse secondary metabolites, including phenolics, flavonoids, tannins, and saponins, which are associated with antimicrobial, antioxidant, and anti-inflammatory properties. This study aimed to develop an antifungal liquid soap incorporating a hydroethanolic leaf extract from a plant species of the Amaranthaceae family, and to evaluate its antifungal potential, cytotoxicity, antioxidant activity, ocular irritancy, and formulation stability. Phytochemical screening was performed by UV-Vis spectrophotometry. Cytotoxicity was assessed in fibroblast cells NIH 3T3) using the MTT assay (50-1000 µg/mL). Antifungal activity against *T. rubrum* was evaluated on PDA medium by inhibition zone assay (extract at 0.5-2.0%). Antioxidant capacity was measured using the DPPH assay (500-2000 µg/mL), and EC₅₀ was calculated. Statistical analyses were performed by ANOVA followed by Tukey's test ($\alpha = 0.05$). Ocular irritancy was tested using the HET-CAM method. pH and organoleptic stability were monitored over 30 days. The extract showed relevant levels of total phenolics (33.72 mg/g), hydrolyzable tannins (40.42 mg/g), condensed tannins (11.19 mg/g), and flavonoids (0.60 mg/g). No cytotoxicity was observed at any concentration; in some cases, the extract significantly increased cell viability ($p < 0.05$). All tested concentrations inhibited *T. rubrum* growth. The extract exhibited dose-dependent antioxidant activity: $25.32 \pm 1.81\%$ (500 µg/mL), $79.34 \pm 1.82\%$ (1000 µg/mL), and $81.47 \pm 1.21\%$ (2000 µg/mL), with EC₅₀ of 240 µg/mL. No ocular irritation was detected, and the formulation remained stable throughout the analysis period. The findings support the potential use of the plant extract as a safe, effective antifungal and antioxidant agent in topical formulations for the treatment of dermatomycoses.

Palavra(s) chave(s): Antifungal; Antioxidant; Soap; Plants.

EUCALYPTUS ESSENTIAL OILS AND EUCALYPTOL: IMPACT ON CYTOKINE REGULATION AND SARS-COV-2 ANTIVIRAL ACTIVITY

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Introduction: SARS-CoV-2, the etiological agent of COVID-19, has established itself as a pathogen of great relevance to global health. In light of the rapid spread of the disease and the scarcity of effective treatments to halt its progression, interest in compounds with therapeutic potential has grown, including natural products derived from plants traditionally used against various ailments. In this context, volatile compounds from the Eucalyptus genus have been studied for their potential therapeutic properties. **Objective:** To evaluate the virucidal and anti-inflammatory potentials of the essential oils from Eucalyptus staigeriana, E. exserta, and E. leptophleba, as well as their main constituent, eucalyptol, against SARS-CoV-2 and cytokines related to COVID-19 in vitro. **Methodology:** leaves from the selected species were collected for the extraction of essential oils and the eucalyptol was commercially purchased. The major constituents were identified by gas chromatography coupled with mass spectrometry. Virucidal activity was assessed using the TCID₅₀ assay, while cytotoxicity was determined in VERO-81 and BEAS-2B cells. The quantification of pro-inflammatory cytokines was performed by flow cytometry. **Results:** Non-cytotoxic doses and the working concentrations for the assays were determined. Chemical characterization revealed a predominance of α -pinene, (-)- β -pinene, o-cymene, D-limonene, eucalyptol, γ -terpinene, and terpinolene. In the virucidal assays, none of the essential oils or the isolated compound exhibited an effect on SARS-CoV-2 at non-cytotoxic concentrations. However, a statistically significant reduction was observed in the production of four pro-inflammatory cytokines (IL-6, MCP-1, IL-8, and CXCL-10). **Discussion:** The chemical composition of essential oils varied from one species to another. The lack of virucidal effect diverges from previous studies with SARS-CoV-2 for the Eucalyptus genus, however, each species synthesizes its compounds both quantitatively and qualitatively, which may influence the observed effects. On the other hand, the reduction in the expression of cytokines associated with the progression of COVID-19 reinforces the potential of these compounds in modulating the inflammatory process, encouraging further in vivo investigations. **Conclusion:** Although the essential oils and eucalyptol did not exhibit a direct effect on the virus, their action in regulating pro-inflammatory cytokines suggests potential as complementary therapeutic agents in the treatment of COVID-19, justifying additional studies.

Palavra(s) chave(s): 1,8-cineol Immunomodulation; Anti-inflammatory Agents; Cytokine Storm; Antiviral COVID-19.

STUDY OF DIAGNOSTIC ACCURACY USING THE FREELITE® METHODOLOGY IN RELATION TO RESULTS OBTAINED BY SERUM PROTEIN ELECTROPHORESIS

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Approximately 2,600 cases of Multiple Myeloma (MM) were diagnosed in Brazil between 2013 and 2019, the Monoclonal Gammopathy of Undetermined Significance (MGUS), often asymptomatic, can progress to MM or other diseases, while Amyloidosis is characterized by the accumulation of free light chains (FLCs) in vital organs. The study evaluated the accuracy of the Freelite® test compared to Serum Protein Electrophoresis (SPE) in detecting monoclonal gammopathies. The SPE tests were carried out on the G26 Easyfix automated analyzer. The research was carried out at the Clinical Hospital of the Botucatu Medical School, and included 203 patients treated in the Hematology, Nephrology, Internal Medicine, Rheumatology and Onco-Hematology sectors. The samples analyzed were subjected to the SPE and Freelite® tests, allowing the comparison of the methods. The data used for statistical analysis were the kappa and lambda indices, together with the K/L ratio in relation with the percentage value of beta and gamma globulins. The results showed that MM was the most prevalent diagnosis (82 out of 203 patients), followed by MGUS (14 out of 203). Amyloidosis was present in only one case. The gender distribution was balanced (49.75% females and 50.25% males). Regarding the effectiveness of the tests, using the Spearman statistic method, for patients with MM, Freelite® showed a statistically significant value only for the correlation between lambda (Freelite®) and gamma globulins (SPE), with a positive Spearman coefficient, that is, as one of the indices increases, the other tends to increase; in MM, SPE detected 24.3% of the cases with a monoclonal peak in gamma, however, Freelite® was able to detect alterations in 25 cases where the SPE was within the reference values. Once a patient's monoclonality has been defined, it is possible to monitor it, based on the iFLC (Involved Free Light Chain), monitoring the results and progression of the disease according to its variation. In patients with MGUS, 28.6% had a monoclonal peak in Gamma; however, the statistical analysis indicated a correlation between Betaglobulins (SPE) in relation to Kappa and Lambda; however, the Spearman coefficient was a negative value, indicating a decreasing relationship. A limitation of this study was the predominance of patients with MM under treatment during follow-up. This characteristic made it difficult to assess the efficacy of the tests in the initial diagnosis. Previous studies have already indicated that SPE has limited sensitivity to detect gammopathies, especially MM. The Freelite® test, by directly quantifying FLCs, increases diagnostic confidence and allows early detection. In conclusion, Freelite® has an adequate performance in monitoring monoclonal gammopathies due to its speed of analysis. The literature recommends the combined use of both methods to ensure a more sensitive and early diagnosis, contributing to a more effective clinical management of patients.

Palavra(s) chave(s): Monoclonal Gammopathies; Free Light Chain Protein; Electrophoresis.

MOLECULAR TESTING IMPLEMENTATION FOR CHLAMYDIA AND GONORRHEA IN REDE NACIONAL DE CARGA VIRAL: A PREVALENCE STUDY IN UNITS SUPPORTED BY THE MOLECULAR BIOLOGY LABORATORY/HC-FMB

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In early 2023, the Rede Nacional de Carga Viral e Hepatites of the Brazilian Ministry of Health implemented molecular detection of Chlamydia (CT) and Gonorrhea (NG) as a complementary test for patients attending the network. The test may be requested during medical consultations in the following situations: HIV diagnosis, use of PrEP, prenatal care, and other conditions where the physician deems it necessary. As the Molecular Biology Laboratory at the Hospital das Clínicas de Botucatu, is part of this network, we aimed to evaluate the adherence of healthcare services in the region to the test, as well as the prevalence of these sexually transmitted infections (STIs) among patients. For this purpose, data were collected on the city of origin and the gender of patients between May 2023 and March 19, 2025. The occurrence of Chlamydia (CT), Gonorrhea (NG), and co-infections was evaluated relative to the total number of patients served, by year and gender. Additionally, cases were analyzed based on the type of sample collected (urine or anal, oropharyngeal, endocervical, and vaginal swabs) received in 2024 and 2025. The results were expressed as percentages. It was observed that adherence to the test by health units outside of Botucatu showed significant growth starting in 2024. During the study period, the prevalence of positive cases was 3.43% in 2023, 5.83% in 2024, and 8.57% in 2025. The prevalence of Chlamydia was 2.94%, 3.58%, and 4.29%; Gonorrhea was 0.49%, 0.67%, and 3.04%; and co-infections were 0%, 1.58%, and 1.25% in 2023, 2024, and 2025, respectively. Chlamydia cases were more frequent in vaginal, endocervical, and anal swab samples, while Gonorrhea was more prevalent in oropharyngeal swab samples. In urine samples, the distribution was homogeneous, with a slight increase in co-infection cases. A higher prevalence of Chlamydia was found in females, while Gonorrhea or co-infection was more prevalent in males. Epidemiological data on these STIs are scarce, particularly in the Brazilian population. However, the literature reports a higher recurrence of Chlamydia in women, while other studies show a greater prevalence of Gonorrhea in men, which corroborates our findings. The higher occurrence of Gonorrhea in oropharyngeal samples could be a laboratory finding from screening asymptomatic patients, as transmission through the oropharyngeal route is less common. For other sample types, our data align with existing literature. Our study reveals good adherence to the molecular CT/NG test by healthcare services supported by the laboratory and underscores the importance of screening for these STIs, as many patients may remain asymptomatic while transmitting the infection.

Palavra(s) Chlamydia; Gonorrhea; Molecular Diagnosis; Ministry of Health; Sexually
chave(s): Transmitted Infections; Screening.

EVALUATION OF THE ANTIOXIDANT ACTIVITY OF LEMON WASTE HYDROLATES: EFFECT OF SOLID-STATE FERMENTATION WITH ASPERGILLUS NIGER

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Citrus fruit peels are often underestimated waste but have great potential for utilization due to their composition rich in bioactive components. Exploring these residues is essential to minimize their improper disposal

in the environment and to obtain these elements of interest. One way to explore them is by extracting the hydrolate and fermenting these residues. Hydrolate is the aqueous fraction obtained in the hydrodistillation process, in this case, from the frit (outer layer of the peel) of Sicilian lemon, containing a small amount of essential oil. Typically, citrus peels are subjected to hydrodistillation to extract essential oils; however, the resulting hydrosols are underutilized. Solid-state fermentation (SSF) uses a natural raw material as a carbon and energy source to promote the growth of microorganisms, usually fungi, on the surface of the solid, moist substrate, with sufficient moisture to support their growth. To produce the hydrolates, 100 grams of lemon frit, provided by JBT Food Tech, were placed in a round-bottom flask with deionized water and subjected to hydrodistillation for two hours using a Clevenger apparatus. The hydrodistillation was performed in triplicate, and the hydrolates were collected and stored frozen until analysis. The objective of this study was to evaluate the effect of solid-state fermentation on antioxidant activity by DPPH and phenolic compounds in lemon frit hydrolate. The results for the hydrolates from raw and fermented residues indicated a 3.03% and 7.23% reduction of the DPPH radical, respectively, and 3.95 and 9.70 µg GAE/mL of phenolic compounds, respectively. Statistically, a significant difference was observed only in the phenolic analysis. This suggests that other factors may influence the antioxidant activity of the sample, such as the chemical structure of the compounds or the presence of other volatile antioxidants. It is necessary to analyze by chromatography whether fermentation may have favored the formation of new metabolites that directly impacted the phenolic content. Solid-state fermentation (SSF) was effective in increasing the phenolic content in lemon frit hydrolate, highlighting its potential for the valorization of citrus residues and the production of bioactive compounds. In conclusion, the study of hydrolates, especially those obtained from citrus residues, emerges as an innovative approach for the sustainable valorization of these residues and the expansion of their biotechnological potential.

Palavra(s) Hydrolate; Residues Lemon; Bioactive Compounds; Solid State
chave(s) Fermentation; Antioxidant Activity.

ANALYSIS OF THE GLOBAL PROBLEM OF CELL LINE AUTHENTICITY BASED ON THE ICLAC INTERNATIONAL REGISTRY OF COMPROMISED CELL LINES

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Introduction: Cell lines are essential tools for scientific research and technological development across multiple fields of knowledge. However, cases of cross-contamination and misidentification of cell lines compromise the fidelity, reproducibility, interpretation, and application of results obtained from experiments involving compromised cells. This issue is global and has been recognized by the scientific community for decades. **Objective:** To provide insights into mitigating the negative impacts of using compromised cell lines in scientific research and biotechnological applications, this study aims to describe the main characteristics of cell line authenticity issues based on the international registry of misidentified cell lines maintained by the International Cell Line Authentication Committee (ICLAC). **Methodology:** We analyzed data on compromised cell lines included in version 13 of the ICLAC registry, published on the

Committee's website on April 26, 2024. Information from each cell line in this database was used to generate additional categorization data, considering both the presumed and verified details about the cells, including organism of origin, embryonic derivation, histological type, malignancy status, and other parameters. The data were organized in an electronic spreadsheet and subjected to descriptive statistical analysis. Results and Discussion: The database analyzed contains 545 records of cell lines classified as contaminated or misidentified. Among these, 321 (58.9%) exhibited discrepancies between the declared and actual cell type. Carcinoma-derived cell lines, particularly those originating from the breast (31; 5.3%), prostate (18; 3.3%), lung (18; 3.3%), papillary thyroid carcinoma (17; 3.1%), and ovarian carcinoma (16; 2.9%), are among the most frequently compromised. The HeLa cell line (cervical adenocarcinoma) was identified as the most common contaminant (141; 25.9%). Of the 497 cell lines originally designated as human, 71 (13.03%) were actually derived from other organisms. Furthermore, 285 (52.29%) records displayed histogenetic discrepancies, including changes in the embryonic germ layer of origin. Conclusion: The available results reinforce the importance of rigorous cell line verification to ensure the reliability of data generated from these cells. Strategies such as strict quality control, periodic genetic authentication, and the use of appropriate cell line sources—such as biorepositories with robust quality control protocols—are essential to mitigate this problem.

Palavra(s) chave(s): Authenticity; Contamination; Cell Lines.

INCIDENCE OF P190 AND P210 TRANSCRIPTS IN CHRONIC MYELOID LEUKEMIA IN PATIENTS TREATED AT THE CLINICS HOSPITAL OF THE BOTUCATU MEDICAL SCHOOL

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Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the presence of an acquired mutation that affects hematopoietic stem cells. In approximately 90% to 95% of cases, the Philadelphia chromosome is expressed, resulting from a reciprocal translocation of the long arms of chromosomes 9 and 22. This leads to the formation of the chimeric BCR-ABL gene, which generates the expression and subsequent translation of an oncoprotein with increased tyrosine kinase activity, p210BCR-ABL, a hallmark of CML patients. For diagnosis confirmation and treatment monitoring, real-time polymerase chain reaction (RT-qPCR) molecular analysis is used due to its high sensitivity. This method allows the identification of BCR-ABL rearrangement and quantification of mRNA copies produced by p210BCR-ABL, proving highly useful throughout therapeutic intervention. It serves as an excellent marker for early assessment of CML treatment response. This study aimed to evaluate the incidence of P190 and P210 transcripts in patients treated at the Molecular Biology Laboratory of the Hospital das Clínicas in Botucatu, Brazil. The study was conducted by collecting data from 665 tests, including patient sex and age, performed for BCR-ABL P190 and P210 diagnosis and monitoring between June 2022 and December 2024. The results showed an incidence of 76%, 90%, and 85% for the P210 transcript in 2022, 2023, and 2024, respectively. The 50 to 59 age group had the highest number of diagnoses, accounting for 25% of cases. Regarding sex distribution, 60% of cases were male. The age group with the highest number of patients undergoing treatment monitoring was 60

to 69 years, representing 28% of cases. The higher prevalence of the P210 transcript in CML, compared to P190, is related to differences in the breakpoint regions of the BCR gene and the specific signaling pathways activated by each isoform, influencing the type of leukemia that develops. The predominance of male patients aligns with national and international epidemiological data, which indicate a higher incidence of CML in men. The increased incidence of CML cases in patients over 50 years old is associated with aging, which leads to a decline in hematopoietic stem cell function, a crucial factor in leukemogenesis, as well as genetic mutations and exposure to ionizing radiation. Monitoring the BCR-ABL gene is essential for studying CML progression, as it determines transcript presence and helps track how patients respond to treatment. Thus, this study contributes to a better understanding of the incidence and distribution of CML, assisting in improving diagnostic and therapeutic strategies for this myeloproliferative neoplasm.

Palavra(s) Leukemias; Chromosome; Molecular diagnosis; BCR-ABL; Incidence;
chave(s): Transcripts P210 and P190.

THE CORRELATION OF VISUAL SKIN ASSESSMENT AND SUBEPIDERMAL MOISTURE EVALUATION IN IDENTIFYING POSTOPERATIVE PRESSURE ULCERS

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Patients undergoing surgical procedures remain immobile and are unable to feel pain or discomfort from the pressure exerted on their skin, preventing them from repositioning to relieve pressure. Additionally, they are often placed on relatively hard surfaces(1). The time required for a pressure ulcer (PU) that develops on a patient's skin to be associated with a surgical procedure varies in the literature, ranging from 48(1-3) to 72 hours postoperatively(1,4). In the early stages of PU development, inflammation caused by tissue damage increases blood flow to the affected area, leading to the accumulation of fluid beneath the epidermis, known as subepidermal moisture (SEM). The increase in vascular permeability results in fluid buildup in the extravascular space, consequently leading to edema, which is not visually detectable in the initial stages of PU formation. SEM can serve as a biomarker for the early identification of PU development(5). Thus, the aim of this study is to evaluate the correlation of Visual Skin Assessment (VSA) and SEM evaluation in identifying postoperative pressure ulcers. This is a cross-sectional, descriptive study with a quantitative approach, conducted at a Tertiary Hospital in the interior of São Paulo, Brazil. The study included 109 patients of both sexes, aged 18 years or older, who underwent surgical procedures across all specialties, performed on weekdays during daytime shifts (7:00 AM to 7:00 PM), with a minimum duration of 2 hours (120 minutes). Data were collected at three time points: the first, second, and third postoperative days. VSA was performed following the guidelines established by the National Pressure Ulcer Advisory Panel (NPUAP)(1) and the SEM measurement was performed using the SEM Scanner®, with four readings per heel (or three readings if the skin had extensive callus formation) and six readings on the sacrum (around the sacral bony prominence). SEM-delta values >0.5 (0.6 and above) were considered abnormal (dichotomous variable)(6-8). SEM-delta abnormalities

persisting for two consecutive days were classified as "tissue damage detected by SEM"(9), referred to as SEM PU. The SPSS software was used to calculate the accuracy, sensitivity, and specificity of SEM in comparison to VSA (the gold standard). The prevalence of PU identified by VSA was 6.4% (n=7), whereas the prevalence of SEM PU was 48.6% (n=53). The accuracy between PU identified by VSA and SEM PU was 52.3%, with a sensitivity of 7.5% and a specificity of 94.6%. These findings indicate that SEM PU prevalence was higher than PU identified by VSA. Although SEM assessment had low sensitivity, its specificity exceeded 90%, suggesting that SEM could be an effective strategy for identifying patients at low risk of developing perioperative PU. This may help optimize physical and human resources in postoperative care.

Palavra(s) Pressure Ulcer; Data Accuracy; Sensitivity; Specificity, Evidence-Based
chave(s): Medicine; Perioperative Care; Biotechnology.

OPTIMIZATION IN FETAL RED BLOOD CELL DETECTION: COMPARATIVE EVALUATION AND OPTIMIZATION WITH MACHINE LEARNING FOR CLINICAL USE

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The detection of fetal red blood cells in maternal circulation is crucial for preventing hemolytic disease of the newborn (HDN), caused by Rh blood incompatibility. Quantification of fetomaternal hemorrhage (FMH) allows for the administration of anti-Rh immunoglobulin, preventing the formation of maternal antibodies that attack fetal red blood cells. Furthermore, accurate detection helps assess the risk of Rh isoimmunization, monitoring sensitized pregnant women, especially those with early-onset pre-eclampsia and fetal growth restriction. The detection of these cells can also contribute to studies of fetomaternal microchimerism. The presence of fetal cells in maternal circulation is a natural phenomenon, but in excess it can be linked to gestational complications. Techniques such as the Kleihauer-Betke (K-B) test and flow cytometry (FC) detect fetal red blood cells, with FC being more sensitive. Machine learning can optimize these techniques, improving detection accuracy and efficiency, contributing to maternal-fetal health. This study, approved by a research ethics committee, recruited pregnant patients with early-onset pre-eclampsia and fetal growth restriction, as well as male volunteers for biological sample collection. Whole blood aliquots were analyzed by flow cytometry, evaluating the co-expression of markers CD235a+ and CD71+ in acquired cells, and by the K-B method. Images of K-B slides were analyzed by a machine learning algorithm. Using the defined gating strategy, preliminary results (7 individuals) showed that FC distinguished samples from pregnant women with negative controls, demonstrating the percentage of CD71+ cells in the analyzed aliquots, and showed correlation with the K-B test results. In a preliminary analysis, the tested algorithm demonstrated potential for detecting and quantifying cells present in the blood smear. The research presents promising results

for the rapid diagnosis of the presented conditions, through the detection and quantification of fetal red blood cells.

Palavra(s) Fetal Cells; CD71 Fetomaternal; Hemorrhage Fetomaternal;
chave(s) Microchimerism; Machine Learning; Rh Isoimmunization.

RADIOLOGICAL PROTECTION IN PET/CT ROUTINE: COUNTING EFFICIENCY OF TWO GEIGER-MÜLLER DETECTORS FOR SURFACE CONTAMINATION MONITORING OF F-18

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Introduction: The use of radiation detectors, such as Geiger-Müller (GM) counters, is mandatory in Nuclear Medicine Services (NMS) for surface monitoring and dose rate measurement. These devices play a fundamental role in radiological safety, assisting in contamination detection and preventing undue occupational exposure. However, the efficiency of these detectors can vary significantly depending on factors such as the radionuclide type (radiation type) and the detection distance. Radionuclides that predominantly emit gamma radiation, such as Tc-99m, exhibit lower detection sensitivity due to the weak interaction of gamma photons with the GM detector gas. In contrast, radionuclides that also emit beta particles, such as I-131, are detected with higher efficiency, as charged particles interact more readily with the detection medium, generating a greater number of ionizations. Objective: To analyze the counting efficiency of two different GM detector models for surface contamination monitoring of F-18 at different distances. Methodology: A liquid F-18 source with an activity of 3,23 µCi at the time of measurement was used, dispersed in a petri dish. Two digital GM detectors were tested: RadEye B20 (ThermoScientific) and Inspector Alert V2 (International Medcom), both calibrated by an accredited laboratory in compliance with regulatory authorities. Measurements were performed by positioning the detectors on stands so that the distances between their sensitive surface and the source were 1.8 cm and 3.8 cm. Ten measurements were taken for each distance. The efficiency of the surface contamination monitor was calculated as the ratio between the average count rate, expressed in counts per second (cps), and the source activity in Becquerel (Bq). Results: For the GM RadEye B20, the counting efficiency for F-18 was 3.6% at 1.8 cm and 1.7% at 3.8 cm. For the GM Inspector Alert V2, the counting efficiency was 3.0% at 1.8 cm and 1.5% at 3.8 cm. Conclusion: The obtained data highlight the importance of considering the detection efficiency of GM detectors for different radionuclides and distances, ensuring active radiological surveillance and compliance with national and international nuclear safety standards.

Palavra(s) Nuclear Medicine; Radiological Protection; Contamination; Positron
chave(s) Emission Tomography; Geiger-Müller.

RADIOLOGICAL PROTECTION IN PET/CT ROUTINE: EVALUATION OF THE EFFICIENCY OF LEAD APRONS FOR RADIATION EMITTED BY F-18

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Introduction: Nuclear medicine is a speciality that uses unsealed radioactive compounds, known as radiopharmaceuticals, for diagnostic and therapeutic purposes. This field stands out for the application of substances that emit gamma or particulate radiation, which are detected by equipment such as Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET). PET, in particular, is one of the pillars of nuclear medicine, playing an essential role in diagnosing oncological, neurological, and cardiovascular conditions. To perform PET scans, it is essential to use positron-emitting radionuclides, such as F-18. These nuclides produce two photons with an energy of 511 keV per decay, which are detected by the equipment. According to the NBR IEC 61331-3 standard, the use of 0.50 mm lead aprons is mandatory for occupationally exposed individuals (OEs) working in radiology services, such as fluoroscopy and prolonged interventional procedures. Additionally, 0.35 mm lead aprons are recommended for OEs in intermediate services, which involve higher exposure procedures. However, due to the high energy levels of radiation emitted by radionuclides used in nuclear medicine, the effectiveness of these lead aprons is a matter of debate. As observed by Ohashi et al., the shielding efficiency of these aprons decreases as radiation energy increases. Objective: To calculate the shielding efficiency of lead aprons with equivalent thicknesses of 0.50 mm and 0.35 mm of lead against gamma radiation emitted by F-18. Methodology: For the measurements and analysis, the Captus 3000 (Carpintec) equipment was used, along with a measuring tape to determine the distance between the detector and the source, and a support to position the source close to the detector and the lead aprons. The measurements were conducted using a point source with 336 µCi of F-18 at the time of acquisition, under the same conditions for both aprons. Results: The tested PPEs showed shielding efficiencies of 3.87% and 3.50% for the 0.50 mm and 0.35 mm lead equivalences, respectively. These results indicate that as radiation energy increases, the effectiveness of these aprons in protecting against gamma radiation decreases. This observation aligns with the study by Ohashi et al., which analyzed the radionuclides Tc-99m, I-131, and Ba-133, with energies of 140.5 keV, 336 keV, and 302 keV, respectively. In the case of F-18, which emits gamma radiation at 511 keV, lead aprons provide negligible protection. Conclusion: Due to their weight and the false sense of protection they may create, the use of lead aprons should not be recommended for routine PET scans in nuclear medicine.

Palavra(s) chave(s): Nuclear Medicine; Positron Emission Tomography; Personal Protective Equipment.

**EXTRACT FROM THE FRUIT RESIDUE OF CARYOCAR BRASILIENSE
CAMB.: A POTENTIAL COMPLEMENTARY TREATMENT FOR
HEPATOCELLULAR CARCINOMA**

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Due to its incidence and severity, hepatocellular carcinoma (HCC) currently represents a major global public health concern. The worldwide incidence is estimated at 1,000,000 new cases per year, with approximately 700,000 deaths. Although treatment is systemic, current chemotherapeutic agents face resistance and fail to halt disease progression. Therefore, complementary therapies using medicinal plants rich in secondary metabolites are gaining relevance. The pequi fruit (*Caryocar brasiliense* Camb.), native to the Cerrado biome and at risk of extinction, is known for its high content of secondary metabolites. Thus, the use of its fruit residue may contribute to both therapeutic development and biodiversity preservation. This study aimed to evaluate the antioxidant potential of the pequi, and assess cytotoxicity in normal fibroblast cells (NIH 3T3) and hepatocarcinoma cells (HUH 7.5). The PFE was prepared using a 1:10 (w/v) ratio of dried peel and outer mesocarp in 70% hydroalcoholic solution. Phytochemical analysis was performed by UV-Vis spectrophotometry using Folin-Ciocalteu for total phenols (TP), aluminum chloride for flavonoids (FL), $\text{CoCl}_2/\text{H}_2\text{SO}_4$ for saponins (SP), and vanillin/ H_2SO_4 for condensed tannins (CT). Antioxidant activity (AX) was measured using lipid peroxidation (LP) and DPPH assays at concentrations of 100, 200, 300, and 600 $\mu\text{g/mL}$, with quercetin (40 $\mu\text{g/mL}$) and saline as positive and negative controls. Cytotoxicity (CT) was evaluated using the MTT assay at 24, 48, and 72 h in NIH 3T3 fibroblasts, and antineoplastic activity (AP) was tested in HUH 7.5 cells using 100-1600 $\mu\text{g/mL}$ of PFE in quintuplicate. Tween 80 (2%) and doxorubicin (2 μM) served as positive controls, while saline was the negative control. For 100 g of dried extract, the phytochemical contents were: TP - 15.19 ± 1.62 mg gallic acid equivalents; FL - 0.0 mg quercetin equivalents; SP - 46.81 ± 3.8 mg saponin equivalents; CT - 21.27 ± 3.4 mg proanthocyanidin equivalents. In the LP assay, inhibition values were: 100 $\mu\text{g/mL}$ - $23.63 \pm 0.01\%$; 200 $\mu\text{g/mL}$ - $34.02 \pm 0.03\%$; 300 $\mu\text{g/mL}$ - $38.53 \pm 0.05\%$; and 600 $\mu\text{g/mL}$ - $47.59 \pm 0.06\%$, compared to $93.89 \pm 0.05\%$ for the positive control ($p < 0.05$ for all treatments vs. negative control). The PFE showed: (1) high levels of saponins and condensed tannins; (2) antioxidant activity; (3) no cytotoxicity in NIH 3T3 cells; and (4) cytotoxicity against HUH 7.5 cells, similar to or exceeding that of doxorubicin. The observed effects are likely associated with its major secondary metabolites. These findings support further investigation into the potential development of a complementary therapy for HCC and the valorization of the pequi fruit and the endangered Cerrado biome.

**Palavra(s)
chave(s):** Antioxidant; Saponins; Plants.

INNOVATION IN THE DETECTION OF EMERGING ARBOVIRAL INFECTIONS AND FIELD MOSQUITO CHARACTERIZATION

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Arboviruses are RNA viruses causing diseases in humans and animals. Their emergence or reemergence, driven by environmental changes, increases mosquito populations and promotes viral spread. Identifying viruses in mosquitoes is critical for understanding transmission cycles and guiding control strategies. Combining detection methods in field-collected mosquitoes with next-generation sequencing (NGS) is key to addressing the growing threat of these viruses, enabling effective epidemic responses and protecting global public health. Objectives: To standardize and identify six arbovirus genera (Phlebovirus, Orthonairovirus, Orthobunyavirus, Alphavirus, Orthoflavivirus, and Thogotovirus) in field-collected mosquitoes. Methods: 343 mosquitoes were collected from four Rio de Janeiro parks (Campo de Santana, Parque Lage, Quinta da Boa Vista, and Chico Mendes) using an electric aspirator. Mosquitoes were photographed, and wings were preserved for morphological analysis. DNA and RNA were extracted individually using the "Extracta 96" device. DNA barcoding, with primers amplifying a 735 bp region of the mitochondrial COI gene, was used for morphological confirmation. The region was divided into two smaller amplicons (COIA and COIB) for Illumina sequencing. Virus detection was performed using RT-qPCR with genus-specific primers, followed by NGS preparation. Results: Bioinformatics tools (FastQC, Trimmomatic, Kraken2) were used for quality control and metagenomic analysis. Nine Orthoflavivirus groups were identified, including *Culex flavivirus* (66.3%), *Orthoflavivirus careyense* (9.0%), *Karumba virus* (9.0%), *Aedes flavivirus* (4.5%), *Kamiti River virus* (4.5%), *Orthoflavivirus cacipacoreense* (2.2%), *Quang Binh virus* (2.2%), *Orthoflavivirus nilense* (1.1%), and *Culiseta flavivirus* (1.1%). Insect-specific viruses, pathogenic viruses, and bacteriophages were detected. Of 270 mosquitoes with successful COI amplification, 74% were *Culex*, 20.5% *Aedes*, 3.3% *Anopheles*, and 0.5% *Uranotaenia* and *Aedini*. Non-insect-specific viruses, such as *Orthoflavivirus cacipacoreense* and *Orthoflavivirus nilense*, were detected in *Culex quinquefasciatus* and *Culex pipiens*, respectively. *Orthoflavivirus careyense* was found in *Aedes albopictus*, *Culex declarator*, and *Culex quinquefasciatus*. Discussion: Insect-specific viruses represented 87.9% of the Orthoflaviviruses found, while 12.1% were non-insect-specific. Studying insect-specific viruses is important for understanding potential interactions with pathogenic viruses in mosquito hosts. Conclusion: The study highlights mosquito viral diversity and the importance of advanced methods like NGS. Initial RT-qPCR screening reduced costs by sequencing only potentially infected mosquitoes. This approach supports epidemiological studies to understand vector-host relationships, contributing to more effective control strategies and protecting public health against arboviruses.

Palavra(s) Arboviruses; Viral Detection; RT-qPCR; Next-generation Sequencing
chave(s): (NGS); Mosquito Vectors; Epidemiological Surveillance.

THE UNIVERSITY AS A GATEWAY TO BIOTECHNOLOGY IN HIGH SCHOOL: A REPORT ON EXPERIENCE

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Introduction: in the recently implemented Brazilian high school curriculum, the domain of natural sciences and its technologies is characterized by a multidisciplinary approach. However, the subject of biotechnology is still infrequently addressed in the curriculum. When it is included, the content is predominantly theoretical, failing to reflect the practical experiences of the majority of students. Objective: this study sought to highlight the importance of direct contact of high school students with biotechnology and its demystification. Methods: a group of high school students who had previously encountered biotechnology in the form of lectures and projects were surveyed to provide a report on their experiences. Results and discussion: with a better knowledge of biotechnology and its possibilities, the students were challenged to develop an innovative technological product with social applicability. They designed a device that uses various technologies such as Global Positioning System (GPS), artificial intelligence, and environmental recognition (unevenness and objects) to help visually impaired people move around independently. This project was presented in a national technology competition (Inova Jovem 2024), which drew over 400 competitors and saw the students' project selected as one of the six finalist teams. After the positive impact of the competition, one of the students participated in another national project, the Technovation Summer School For Girls, which targeted exclusively female students aged 8 to 18. The aim of this project is to develop another technology with social applicability, with a view to participating in an international competition, Technovation Girls. The implementation of both projects has facilitated the enhancement of students' scientific and technological knowledge, fostering the development of teamwork, social and professional responsibility, and entrepreneurship skills. Conclusion: in this context, it is evident that practical engagement with biotechnological development during high school not only facilitates students' exposure to science and the entrepreneurial sector as domains within reach of all, but also exerts a favorable influence on these domains and on the development of the next generation. In light of these observations, it is recommended that the university consider the incorporation of university extension within the postgraduate curriculum as a means of enhancing the accessibility of biotechnological knowledge to a broader range of students from diverse social backgrounds. Consequently, while the university would essentially be extending its existing programs, the students would encounter an expanded array of prospects, a compelling motivation, and a valuable opportunity.

Palavra(s) Biotechnology; High School; University Extension; Entrepreneurship;
chave(s): Innovation.

GRAPHENE ELECTRODES: SYNTHESIS, PROPERTIES, AND POTENTIAL FOR DIAGNOSTIC APPLICATIONS

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Graphene stands out for its excellent intrinsic properties (high conductivity and chemical stability, high mechanical strength, etc.). These characteristics make graphene a highly relevant material for scientific, industrial and medical applications. In addition, the diversity of graphene applications is due to the changes in its intrinsic properties obtained from interfacial modifications or "defects" created in its structure, whose properties can be adjusted for a specific application, such as in the production of biosensors. Graphene-based biosensors offer high sensitivity and excellent biocompatibility, allowing biomarkers to be identified in extremely low concentrations, which makes it possible to make compact, portable, low-cost diagnostic devices that are ideal for field diagnosis. In view of this, the aim of this work was to verify the best conditions for printing stable graphene electrodes with maximum conductivity and good flexibility, in order to make future modifications for application in biosensors. To manufacture the graphene electrodes, a polyimide substrate reinforced with Polaseal was used as the basis for the printing process. The technique involves using the VISUTEC Laser Cutting and Engraving machine model VS6040 (60W, 10600 nm) to carbonize the polyimide using a high-power laser, which generates a plasma and, through the ablation process, promotes the formation of graphene on the surface of the material. During printing, controlled environmental conditions were applied and different printing conditions were tested, varying the power between 8.5% and 10.2% and laser speed, while the electrical resistance was monitored using a multimeter to optimize the production of the electrodes and guarantee characteristics such as homogeneity and conductivity. After carbonization, the samples were coated with adhesive to protect the contacts and delimit active areas. These tests made it possible to assess the conductivity and resistance of the material produced. At the end of the project, it was possible to determine the best printing conditions which resulted in the production of electrodes with excellent electrical conductivity, structural homogeneity and mechanical resistance, ensuring the durability and performance of the devices. This study investigated the use of graphene to produce electrodes with high conductivity, flexibility and reproducibility, with a view to future applications in biosensors. The results showed that graphene is ideal for electrodes, as its intrinsic properties, combined with the control of parameters such as environmental conditions, laser power and speed, resulted in devices with superior conductivity, structural uniformity, mechanical resistance and no peeling or degradation. These characteristics highlight graphene as a promising material for applications in biosensors and diagnostic devices.

Palavra(s) chave(s): Polyimide; Graphene Electrodes; Biocompatibility; Biosensors.

EVALUATION OF SOFT SKILLS OF MEDICAL STUDENTS

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The rapid evolution of technology has impacted all sectors, and health is no exception. Today, Medicine 4.0 stands out as an innovative trend, driven by the development and use of new approaches and technologies. The World Health Organization (WHO) defines Health 4.0 as a field that uses these technologies to improve health. This concept broadens the idea of Health, including digital consumers and smart devices connected to the internet. In addition, it covers technologies such as Internet of Things (IoT), Artificial Intelligence (AI), Big Data and Robotics. These innovations are transforming the medical landscape, providing new tools and opportunities for diagnosis, treatment and patient care (CAPUCHO, 2021). Medicine 4.0 is linked to the evolution of medical knowledge and the development of advanced technologies, where digital systems operate in an integrated way, based on communication and collaboration, which facilitates the processes of monitoring and patient care (PEREIRA; RIBEIRO, 2021). To succeed in this new context, health professionals will need to develop more complex and creative cognitive skills, be able to analyze information, make quick and effective decisions, and adapt to a constantly changing environment (OLIMPIO NETO, 2022). Seeking innovative solutions and thinking creatively is essential to meet the challenges that the future of medicine presents, as well as the willingness to work in teams and multidisciplinary groups. The objective of this study was to evaluate the soft skills of students in the second year of medical school enrolled in the optional discipline Behavioral skills in the professional sphere. Applied research was developed, of quantitative and exploratory nature, focusing on six fundamental skills for professional performance in medicine: risk acceptance, creativity, teamwork, resilience, leadership and interpersonal relationships. The behavioral assessment tests were applied at the beginning of the course, through a form in Microsoft Forms, and the answers were analyzed using a five-point Likert Scale, with the supervision of an organizational psychologist. Overall, the soft skills of the class remained at a good level, however, the skills that obtained the best result were creativity, resilience, leadership and interpersonal relationships where 68.33% are at a good level and 30% at an excellent level, while in the skills acceptance of risks and teamwork, 43.33% of the students remained at a satisfactory level and only 40% at a good level. Soft skills are not innate and can be developed through a continuous commitment to personal and professional growth (CODA, 2023). Thus, the results indicate that the improvement of risk acceptance and teamwork skills will need the student's engagement in their own transformation.

**Palavra(s)
chave(s):** Entrepreneurship; Higher education; Medicine 4.0; Soft Skills.

BOVINE PENIS: SAFETY OF OBTAINING AT THE SLAUGHTERHOUSE AND MICROBIOLOGICAL CONTROL IN USE FOR DOG FOOD

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The safe acquisition of animal by-products in slaughterhouses is a challenge to ensure the microbiological quality and commercial viability of these products. This study analyzed the production chain of bovine penis, from its acquisition at slaughter to its processing for the manufacture of dog chews, with an emphasis on sanitary and microbiological safety. The research on the process of obtaining was realized in a Brazilian slaughterhouse with Federal Inspection Service, located in the state of São Paulo, where the stages of slaughter involving the removal, cleaning

and storage of the product were evaluated, such as extraction, washing, draining, packaging and freezing. In the subsequent processing to obtain the chewable product for dogs, different biotechnological strategies were tested, such as hydrogen potential (pH) correction by immersion in an acid solution, controlled drying in three thermal curves and sterilization. The microbiological safety of the final chewable product was monitored over six months in an accelerated shelf-life study, with analyses for *Salmonella* spp., *Escherichia coli* and Mesophilic Aerobes. The results demonstrated that correct extraction during slaughter, associated with an optimized processing protocol, guarantees a safe and stable final product, without the presence of pathogens and with a significant reduction in the microbial load over time. The pH of the rod before the acid bath showed an average value of 7.03 and after the bath it decreased to 5.68. The drying curve at 65°C proved to be more effective, limiting the growth of pathogenic microorganisms, since during the entire study period there was no detection of *Salmonella* spp, the colony forming unit (CFU) count for *Escherichia Coli* was less than 10 and that of Mesophilic Aerobes decreased from 10^4 at the beginning to less than 10 at the end of the period, probably due to the low water activity of the chewable (0.55). This study reinforces the importance of biotechnology applied to safety in obtaining and processing animal by-products, contributing to innovation in the pet industry, reducing waste in the cold chain and valuing previously underused materials.

Palavra(s) chave(s): Bovine; Chewable; Microbiology; penis; Slaughter.

EVALUATION OF THE EFFECTIVENESS OF A NEW PRESERVATIVE SOLUTION FOR USE IN THE HEMATOLOGY LABORATORY

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INTRODUCTION: Quality controls are critical in clinical laboratory analysis. They facilitate the evaluation of analytical processes, ensure compliance with current legislation, and enable the release of reliable results. Ideally, biological samples of quality controls should be standardized and evaluated both intra- and inter-laboratory. However, a limited number of specialized services offering these samples are available in Brazil, and in-house standardization requires validation to maintain stability and reproducibility of analytes over time. **OBJECTIVE:** The objective of this study was to develop control samples using a novel preservative solution for application in external and internal quality control tests in the field of hematology. **METHODOLOGY:** The local CEP committee has approved the present study under the designation 6.265.548. Peripheral blood samples (20 ml) were collected from healthy individuals ($n = 7$) and separated into three aliquots to adjust concentrations for low, normal, and high cellularity levels. The samples were stabilized using the preservative solution, and the hematological parameters were monitored over a 56-day period. To assess the variation over time, the Levey-Jennings graphs and linear regression analysis were adopted and then compared to commercial controls. **RESULTS:** The outcomes demonstrated the effectiveness of the novel preservation solution in the field of hematology, maintaining the stability of erythrocytes and platelets for over 50 days and the complete blood count for up to 21 days for in vitro use. This proof of concept is

significant for two primary reasons. First, it serves to characterize the product's market potential. Second, it facilitates the conduct of external and internal quality assessments. CONCLUSION: These assessments can be utilized for specialized services that produce and commercialize stabilized samples for proficiency assessments. They can also be employed by public or private services that aim to produce their internal controls independently. This proves to be useful in the context of laboratory quality management.

Palavra(s) chave(s): Quality Control; Hematology; Preservative Solution; Quality Assurance.

ADVANCES IN MAGNETIC RESONANCE IMAGING TECHNIQUES FOR SOFT TISSUE TUMORS: A CLINICAL EVALUATION

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The imaging diagnosis of soft tissue tumors has advanced substantially with the emergence of cutting-edge magnetic resonance imaging (MRI) techniques, driven by developments in biotechnology applied in medical imaging. Among them are the notable high b-value diffusion-weighted imaging (DWI) and dynamic contrast-enhanced perfusion (DCE) that significantly increase the enhancement of tissue characterization, the assessment of these aggressiveness and cellularity lesions. DWI enables more accurate evaluation of water molecule movement restriction in tissues, generating apparent diffusion coefficient (ADC) maps that are highly sensitive to cellularity and membrane integrity, often altered in malignant tumors. DCE provides dynamic information regarding tissue vascularization and capillary permeability, with reported sensitivity, specificity, accuracy, and positive and negative predictive values of 81%, 75%, 78%, 76%, and 81%, respectively, for differentiating between benign and malignant tumors. The combination of DWI and DCE has shown to be particularly useful in distinguishing musculoskeletal tumors, increasing diagnostic accuracy up to 90.9%, and is considered a significant improvement over conventional MRI. In this context, DWI and DCE techniques were implemented and standardized in the MRI Department of the Clinical Hospital, Botucatu Medical School (HCFMB), in 2022 for the evaluation of soft tissue tumors. This study aimed to compare the MRI reports for soft tissue tumor characterization—benign versus malignant—with histopathological findings. MRI exams performed between January 2022 and December 2024 were selected, including cases of patients with suspected soft tissue tumors in the upper or lower limbs, who carried out both MRI with DWI and DCE sequences and subsequent histopathological analysis, all performed at HCFMB. According to these criteria, 12 patients were included. All MRI reports were provided by the same radiologist, prior to the histopathological diagnosis, for comparison. The diagnostic accuracy of this study was 91.6%, with concordance observed in 11 out of the 12 selected cases. The adoption of these advanced protocols, made possible by improvements in pulse sequences and post-processing techniques, reflect the growing integration between advanced imaging and biotechnology. This integration has led to a significant enhancement in the qualitative assessment (binary answers such as "yes" or "no") for distinguishing between benign and malignant tumors. Beyond the diagnostic role, these techniques also support biopsy site planning by indicating viable tumor regions, thereby improving

histopathological diagnostic accuracy and facilitating therapeutic planning. Following the implementation of these techniques, a marked improvement was observed in the differentiation of soft tissue tumors in patients treated at HCFMB.

Palavra(s) Soft Tissue Tumors; Magnetic Resonance; Diffusion-Weighted Imaging;
chave(s): Dynamic Contrast-enhanced Perfusion.

IMPLEMENTATION OF A MOLECULAR DIAGNOSTIC TEST FOR THE DETECTION OF HTLV VIRUS

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Introduction: Human T-cell leukemia virus type-1 (HTLV-1) is associated with neurological and lymphoproliferative disorders, while HTLV-2 has not been definitively linked with a specific disease. Because HTLV is transmitted horizontally, since 1993 serological screening has been required for blood donors. Although it is a gold standard methodology, confirmation of positive results is necessary due to the high sensitivity of the tests. Currently, only chemiluminescence-based immunoassays are in use at the Clinical Hospital of The Botucatu Medical School (HCFMB). The implementation of a molecular test is highly relevant to confirm and identify the viral subtype, improving the patient follow-up. Objective: To validate and to implement a qualitative multiplex real-time PCR assay, previously developed by a Brazilian group, for the detection and identification of HTLV-1/-2 subtypes at the HCFMB. Methodology: DNA extraction from leukocytes was performed using an in-house silica column-based method. The viral targets used in the real-time PCR (qPCR) were pol and tax genes. The patients were categorized in four groups. Group 1- Samples from soropositive patients under follow-up at the Specialized Outpatient Service for Infectious Diseases "Domingos Alves Meira" (SAEI-DAM)-Botucatu. The confirmation of the viral subtype was performed by Sanger sequencing using the tax gene for HTLV-1/-2. Group 2- Blood donor samples for evaluation of sensitivity and specificity in comparison to ARCHITECT rHTLV-I/II assay (Abbott Laboratories) in use at the HCFMB Blood Center. Group 3- Samples from HIV positive patients. Group 4- Samples from patients co-infected with HIV, HBV, and/or HCV processed at the Laboratory of Applied Biotechnology (LBA) for viral load testing. Results: Group 1- Four samples were analyzed: HTLV-1 (n=2), HTLV-2 (n=1), and 1 negative. All positive samples were confirmed by Sanger sequencing. Group 2- 75 blood donor samples were collected until February 2025. All samples were negative by serological and qPCR tests, showing a specificity of 100%. For sensitivity tests new samples will be collected. Group 3- No cases of HIV/HTLV co-infection were found (0/34). Group 4- Co-infected samples showed HTLV-1/HIV/HCV (1/30), HTLV-2/HIV/HCV (1/30), HTLV-2/HIV/HBV/HCV (2/30), and negative (26/30) results. Sanger sequencing was used to confirm the viral subtype of positive samples. Discussion: The qPCR confirmed all but one soropositive samples in Group 1. Probably, the exception is a false-positive case, considering the high sensitivity of the method. All HTLV subtypes were confirmed by Sanger sequencing, excluding the interference of HIV and hepatitis virus in co-infected patients. Tests of sensitivity, reproducibility and repeatability are in progress to complete the test implementation at the HCFMB. Conclusion: Preliminary results of our study have shown that

qPCR assay used in this study is useful to confirm and also identify HTLV subtypes.

Palavra(s) chave(s): Molecular Diagnostic Techniques; HTLV-1 infections; HTLV-2 infection.

DEVELOPMENT OF A TRANSDERMAL CONTROLLED-RELEASE MEMBRANE BASED ON CHITOSAN, PLASMA AND PLATELETS FOR SKIN WOUND REGENERATION.

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Chronic wounds represent a major public health challenge, particularly among elderly populations and individuals with diabetes, often resulting in prolonged healing times and increased healthcare costs. The search for effective, accessible, and biocompatible therapeutic alternatives has led to the exploration of natural polymers and blood-derived components. In this context, the present study aimed to develop and characterize transdermal membranes composed of chitosan, fresh frozen plasma (FFP), and platelet concentrate (PC), with the goal of promoting epithelial regeneration in chronic venous ulcers. The membranes were prepared by fusion, incorporating FFP and PC obtained from voluntary donors at the Hemocenter of Botucatu into chitosan matrices crosslinked with diluted acetic acid in three different concentrations, ranging from 3% to 5%. These membranes were physically characterized in terms of their thickness and morphology; mechanically, through tensile tests in accordance with ASTM D882-91; and biologically, using the agar diffusion method. The results demonstrated that the addition of FFP and PC improved the performance of the membranes by increasing adhesion and bioactivity while maintaining structural integrity and biocompatibility. The membranes not only preserved the mechanical properties essential for wound dressing applications but also exhibited significant antimicrobial activity, especially against fungi and bacteria. The images revealed a homogeneous morphology with interconnected porosity conducive to nutrient diffusion and cellular infiltration. Mechanical analysis of tensile strength and elongation percentage of both doped membranes and those composed solely of chitosan showed adequate flexibility and strength for application to skin tissue. These findings suggest that chitosan-FFP and PC membranes are promising candidates for chronic wound treatment, offering a cost-effective, biocompatible, and bioactive alternative capable of promoting tissue regeneration. Future studies should explore in vivo performance and the potential for incorporating additional therapeutic agents. In conclusion, the integration of chitosan, fresh frozen plasma, and platelet concentrate into transdermal membranes presents a viable and innovative strategy to accelerate the healing of chronic wounds and improve the quality of life for affected patients

Palavra(s) chave(s): Chitosan; Blood Products; Healing of Chronic Wounds.

IN-HOUSE MOLECULAR TEST BY REAL-TIME PCR FOR DETECTION OF FACTOR V LEIDEN AND PROTHROMBIN MUTATION

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Introduction: Thrombosis is a serious medical condition that can be caused by several factors, including genetic mutations. The G1691A mutation in factor V Leiden (FVL) and G20210A in prothrombin are two of the most common inherited mutations associated with venous thromboembolism (VTE). FVL mutation elevates thrombosis risk, varying by genotype. Heterozygotes face a 10% lifetime DVT risk (25-40% in thrombophilic families), while homozygotes have a much higher risk. FVL strongly increases thrombosis risk, especially in homozygotes. Prothrombin G20210A Gene Mutation (PGM) increases thrombosis risk, especially in portal and cerebral veins. Carriers have a tripled risk for venous thrombosis and a quadrupled risk for portal vein thrombosis. Commercial tests for these mutations can be expensive, especially in Brazil. Laboratory tests for FVL and PGM are priced at R\$ 256.00 and R\$ 482.00, a costly alternative for patients needing diagnosis. Development of a low-cost inhouse kit may increase accessibility to these tests and contribute to the early diagnosis and treatment of VTE, thereby reducing mortality. **Objectives:** Development of a low-cost in-house kit for detecting the FVL and PGM related to thrombosis by real-time PCR. **Materials and methods:** Peripheral blood mononuclear cells extraction was isolated using an adapted Chomczynski, Piotr; Sacchi, Nicoletta protocol. DNA extraction was performed using the commercial kit Bioclin. The primers and probes used to detect mutations were commercially acquired. For standardization, we set the qPCR for 50 cycles: 95°C for 10 s, 58°C for 45s. Primer concentrations tested for wild-type and mutant FVL and PGM were 200, 400, 600 and 800 nM, while the probes concentrations were 200, 400 and 600 nM. **Results:** Standardization of qPCR for FVL and PGM identified optimal concentrations of forward and reverse primers, and probes, for each allele. For FVL G1691A, the wild-type allele showed better efficiency with 600nM forward primer and 600 nM reverse primer, and 600 nM probe, while the optimized mutant allele was with 400 nM forward primer and 400 nM reverse primer, and 200 nM probe. For PGM, the optimized mutant allele was with 400 nM forward primer and 400 nM reverse primer, and 200 nM probe. However, for PGM, the wild-type allele showed no detectable amplification, even after repeating the assay with multiple samples, suggesting a possible defect in the synthesized probe. **Discussion:** Optimization of primer and probe concentrations was crucial for a sensitive and specific qPCR assay. Identifying the ideal conditions will allow for a low-cost kit for diagnosing thrombophilia, with the potential to improve diagnosis and treatment. **Conclusion:**The standardization of qPCR for FVL and PGM resulted in the identification of the ideal concentrations of primers and probes, enabling a low-cost kit for the diagnosis of thrombophilia.

Palavra(s) chave(s): G1691A; G20210A; Low-Cost Thrombosis; Blood-Coagulation SNP.

DEVELOPMENT OF A TECHNOLOGICAL SYSTEM FOR MANAGING SUPPLIES IN A FLOW CYTOMETRY LABORATORY

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The management of consumables in flow cytometry laboratories is challenging due to the wide variety of reagents, which vary in composition, concentration, expiration, and consumption, requiring strict control to prevent waste, ensure test accuracy, and ensure reagent availability. The process is further complicated by factors such as titration and the monitoring of the monoclonal antibodies performance. The current management system takes a generic approach, without considering the specificities of flow cytometry. The objective of this study was to develop and choose the most suitable software for laboratory inventory control in partnership with SENAI Botucatu and Technical Course in Systems Development. The development process involved the utilization of VS Code (front-end IDE), Spring Tool Suite 4 (Java back-end), and MySQL Workbench (database). The web interface was constructed using HTML, JavaScript, and Bootstrap, while Figma was employed for prototyping. The Spring Boot framework served as the primary web tool for application construction. API testing was conducted using Postman, and project management followed Scrum (bi-weekly sprints) and Kanban methodologies. Version control was implemented using GitHub to ensure collaboration and traceability. Two groups were established to evaluate two different systems (Group A and B). Following a thorough evaluation that encompassed safety criteria, the availability of qualitative and quantitative reports. The Group A system was characterized by its intuitive nature, featuring shortcuts for all functions. The expiration and low stock alerts are clearly marked and include margin of error calculations, facilitating management. However, the system does not generate financial reports and requires manual stock adjustment for expiration, which is susceptible to error. The Group B system was distinguished by its easy-to-use and secure interface, which requires supervisor approval for new entries. The reagent stock is presented in an organized manner, and the system automatically discounts entries that are closest to expiration. However, the panel registration process is intricate, necessitating manual subtraction of tests from the stock. Additionally, there is an absence of financial reports or alerts for low stock. Points for improvement were highlighted for both groups based on the weaknesses of each software, mainly focusing on the financial reports. The selection of the software was determined by observations, with the software from the first group being selected. Even though Group B offers enhanced security, the first group's lean design and intuitive interface were deemed conducive to its selection as the software to be validated. This outcome indicates the initial development of laboratory control software, which is now entering a new phase of development with validation and implementation for routine laboratory use.

Palavra(s) chave(s): Technological System; Quality Control; Quality Management.

APPLICATION OF ARTIFICIAL INTELLIGENCE IN THE AUTOMATIC SEGMENTATION OF ORGANS AT RISK IN RADIOTHERAPY: ADVANCES AND CHALLENGES

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INTRODUCTION: Artificial intelligence has contributed to the automation of various processes in different sectors, including healthcare, and is increasingly being implemented in clinical routines to automate the radiotherapy workflow. Accurate segmentation of organs at risk (OARs) is essential for radiotherapy planning and for limiting treatment toxicity. However, the time spent on this task is significant in high-demand radiotherapy centers. Given this, the use of artificial intelligence to optimize the radiotherapy workflow shows promising future prospects. Therefore, it is important to explore this potential. **OBJECTIVE:** To analyze the advances and challenges of applying artificial intelligence to the segmentation of OARs in radiotherapy. **METHODOLOGY:** A narrative literature review based on the selection of articles published in the last 5 years in the PubMed database, using a research combining the key terms "artificial intelligence" and "radiotherapy automatic segmentation". **RESULTS:** Convolutional neural networks are the main AI techniques used in the automatic segmentation of OARs. Organs with high contrast, such as the eyes, lungs, and bladder, exhibit good accuracy in automatic segmentation, while organs with small volumes and diffuse boundaries, such as the optic chiasm, still pose challenges. The segmentation of OARs is simpler than that of lesions, due to the similarity of OARs across different patients, unlike lesions, which have variable shapes and sizes. Dosimetric evaluations indicate that the automatic segmentation of OARs can be incorporated into clinical practice, with no significant differences in dose-volume parameters compared to manual segmentation. **DISCUSSION:** AI contributes to speeding up the contouring of OARs and reducing the human workload. Most AI-based image segmentations are clinically acceptable, but final verification by radio-oncologists and radiotherapy dosimetrists is still required to validate the process. Time savings and workflow efficiency are the main advances of AI in OARs segmentation. However, challenges such as operational costs, the need for greater segmentation reliability, regulatory gaps, ethical considerations, and the need to train effective AI models must be overcome to ensure the safe and effective adoption of this technology. **CONCLUSION:** Key technological advances include improved performance, accuracy, time savings, and the potential to reduce the workload of radiotherapy teams. However, the application of AI in radiotherapy remains a challenge, as it is still in its early stages and requires further study to ensure greater safety in the application of radiotherapy techniques.

Palavra(s) Advances; Artificial Intelligence; Automatic Segmentation; Challenges;
chave(s): Organs at Risk; Planning Radiotherapy; Radiotherapy.

BUDGETARY IMPACT ANALYSIS FOR THE IMPLEMENTATION OF A NEW METHOD FOR ASSESSING LIVER FIBROSIS: FIBMASTER IN PATIENTS WITH HEPATITIS C IN THE SUS NETWORK

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Introduction: Hepatitis C is a silent viral disease that induces liver damage, which becomes chronic in 80% of the patients, being able to evolve to severe damage, such as cirrhosis and hepatocellular carcinoma. The liver fibrosis evaluation is necessary for these patients, and the biopsy is the gold-standard method, although it is an invasive exam involving risks. Nowadays, non-invasive methods have been stimulated in the monitoring protocols of liver diseases. Thus, FibMaster was recently developed to evaluate liver fibrosis in chronic hepatitis C patients through blood biomarkers. Objectives: This study proposed to analyze the budgetary impact of this new method to be implemented at Brazilian National Healthcare System - SUS (Sistema Único de Saúde). Methods: Our applied methodology was Budgetary Impact Analysis (from Portuguese, Análise de Impacto Orçamentário - AIO), which considers: the analytics model, perspective, reference scenario, alternative scenario, time horizon, target population; direct costs; market behaviour, sensibility analysis, input data and output data. Whence it was surveyed the target population who would benefit from the new method and the comparison between reference scenarios and alternative scenarios in the time horizon of 4 years from the available data in the Procedures Table, Medicines and Orthoses, Prostheses, and Special Material from SUS Management System (from Portuguese, Sistema de Gerenciamento da Tabela de Procedimentos, Medicamentos e Órteses, Próteses e Materiais Especiais - SIGTAP) and administrative contracts. Results and Discussion: It was able to identify that the new method, between 2023 and 2026, presents a saving estimate of R\$ 3.657.917,00 if used in the diagnostic process for liver fibrosis staging in comparison to liver biopsy, and an investment of R\$ 213.097.022,00 if implanted in the hepatitis C patient's escort process, in association with the FIB4 method, which is being currently used. Conclusion: The novel method will provide a non-invasive diagnostic alternative that has a finer diagnostic precision to stages $\geq F2$, great national coverage for using National Public Network for CD4/CD8 T Lymphocyte Counting intended for monitoring people that live HIV/AIDS, for fibrosis stages $\geq F2$ (significant fibrosis), providing flexibility regarding methods in the diagnostic process and liver fibrosis monitoring, contributing to the work teams and enabling a better quality of life for these patients, directly impacting 317,904 people infected with hepatitis C and indirectly 718,651 people affected with viral hepatitis expected until 2026. Furthermore, manual guidance was also developed for applying the AIO methodology aimed at professionals who need to conduct an economic health evaluation.

Palavra(s) chave(s): Budget Impact Analysis; Hepatitis C; Health Economic Evaluation; SUS.

II Workshop Internacional em Biotecnologia na Saúde:

Caminhos da Inovação

ISBN: 978-65-5067-082-5

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