International conference
Cannabis and science X

Book of Abstracts

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Brno Observatory and Planetarium

Welcome note/Brief note

CANNABIS AND SCIENCE – 10 TH. ANNIVERSARY

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INTRODUCTION

The International Conference Cannabis and Science is celebrating its 10th year this year. And we are skipping the year when we were not allowed to meet due to the COVID 19 epidemic. In 2013, legislation allowing the use of medicinal cannabis for treatment came into force in the Czech Republic. The first year was held in 2014 at Mendel University in Brno. Since 2019, it has been organized in cooperation with the Faculty Hospital at St. Anne's in Brno. And for the last five years, we have been meeting at the Observatory and Planetarium on Kraví hora in Brno. This place best describes the topic of cannabis, not only with its environment and size, but above all with its spirit, which is not a topic for a year or ten years, but is a story that has accompanied humanity for thousands of years. And it is precisely to develop knowledge among experts, scientists, doctors, patients and the general public that the conference Cannabis and Science came into being very spontaneously. Today, it is a symbol of the topic of cannabis in the Czech Republic and is one of the regular educational events of the Czech Medical Chamber. This year's novelty is the possibility of presenting scientific papers in the form of a poster, which brings a completely official use of the conference for, for example, high school, master and doctoral students. During its duration, the conference brought dozens of high-quality, internationally recognized scientists from various countries around the world to Brno. Israel, the United States of America, the Netherlands and Poland are just a small taste of the places from which our guests come to us. The conference serves to share new scientific information from the world of cannabis and is not primarily a commercial event. Therefore, support from the commercial sector is very important. Only thanks to cooperating companies can we make the conference accessible to everyone. I would like to thank all partners and, last but not least, visitors for their support and participation in the groundbreaking conference Cannabis and Science. And I firmly believe that the 10th anniversary is just the beginning!

Keywords: Cannabis and Science

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Conference program

Time	Speaker	Topic
9:00–9:20	Opening	
9:20–10:00	Hanuš Lumír	History of cannabis and isolation; Lumir Lab. Jerusalem Biotechnology Park Hebrew University, Israel
10:00–10:30	Rokyta Richard, Fricová Jitka	Pathophysiology of cannabinoids and its clinical application, Department of Physiology, 3rd Faculty of Medicine, Charles University, Prague
10:30–11:00	Bernstein Nirit	Progress in cultivation science for enhanced secondary metabolites and yield in cannabis; Volcani Institute of Agricultural Research, Israel
11:00–11:30	Tjalling Erkelens	Global regulatory and commercial developments in cannabis for medicinal purposes, Bedrocan International BV
11:30–11:50	Mosińska Agnieszka, Dvořáková Barbora	Novel approach in the analysis of phyto- and endocannabinoids in plant and biological samples under in vivo conditions. Medical University of Gdaňsk Poland, Grammar School Brno-Řečkovice
11:50–12:10	Kaszewska Magdalena, Woźniczka Katarzyna	Perspectives of cannabis-based medicines in humans. Optimization of SPME-LC-MS/MS method for analysis of selected endo- and phytocannabinoids in serum samples. Medical University of Gdaňsk Poland
12:10–13:50	Lunch, informal discussion	
13:50–13:55	Trojan Václav	10 let konference
13:55–14:05	Šulcová Alexandra	Therapeutic indications of CBD
14:05–14:30	Landa Leoš	The importance and therapeutic potential of CBD in veterinary care, Institute of Pharmacology, Faculty of Medicine, Masaryk University
14:30–14:50	Klapková Kristýna	Conditions for the use of cannabis plants and extracts in veterinary care from the perspective of legislation – Part I – Institute for State Control of Veterinary Biologicals and Medicines
14:50–15:10	Nepejchalová Leona	Conditions for the use of cannabis plants and extracts in veterinary care from the perspective of legislation – Part II – Institute for State Control of Veterinary Biologicals and Medicines
15:10–15:40	Mravčík Viktor	Psychomodulants, Department of Drug Policy, Office of the Government of the Czech Republic; Podané ruce Society
15:40–16:00	Tarkowski Petr	Where are you heading to, cannabis research? Palacky University Olomouc
16:00–16:20	Halámek Jan	Bioaffinity-based concept for metabolite panel characterization. Institute of Forensic Science, Department of Environmental Toxicology, Institute for Forensic Sciences, Texas Tech University
16:20–16:40	Mištríková Petra	Medical cannabis add – on therapy in patients with trigeminal neuralgia, Neurologická ambulance pro dospělé s.r.o.
16:40–17:00	Dvořák Dušan	Patient and medical cannabis

Speakers

Hanuš Lumír
Rokyta Richard, Fricová Jitka
Bernstein Nirit
Tjalling Erkelens
Mravčík Viktor
Landa Leoš
Klapková Kristýna
Nepejchalová Leona
Mosińska Agnieszka, DvořákováBarbora
Kaszewska Magdalena, Woźniczka Katarzyna
Tarkowski Petr
Halámek Jan
Mištríková Petra

Participants of the poster session

Alena Ryšavá
Jan Dehner
Piotr Graczyk
Stefan Bagieński, Piotr Gołaś
Hanna Grodzka, Martyna Grochowalska

Abstracts

HOW ARE SUBSTANCES ISOLATED FROM CANNABIS AND THE BRAIN?

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INTRODUCTION

Cannabis has been used as a fiber, medicinal plant, and recreational drug since ancient times. Information about this plant dates back thousands of years. The oldest tangible archaeological findings come from the Czech Republic, followed by China. The effort to identify the active substance in cannabis and, after the discovery of cannabinoid receptors, the active substance in the brain dates back to 1821, when the first chemical scientific paper was published.

MATERIALS AND METHODS

The goal of researchers was to isolate and identify the substance responsible for the medicinal and psychoactive effects of cannabis. This was not an easy task, as these compounds are oily rather than crystalline alkaloids, making their isolation in a completely pure form significantly more challenging. As a result, the first isolated cannabinoid was an artifact, cannabinol, in 1940. The first truly natural cannabinoid, the antibacterial cannabidiol acid, was isolated in 1955. The psychoactive compound from cannabis, tetrahydrocannabinol (THC), was identified by two research teams in 1964. Thus, the clarification of the plant's effects was successfully achieved.

Following the discovery of cannabinoid receptors, efforts were made to isolate a substance from the brain that, like THC, binds to these receptors. This was not an easy task, as the compound exists in the brain only in picomolar concentrations. Nevertheless, this challenge was also overcome, and the substance, named anandamide, paved the way for the medical use of cannabis.

RESULTS

The results are promising, research continues, but advancing cannabis for medical treatment is not easy.

CONCLUSION

Cannabinoids and endocannabinoidshavepaved the way for cannabis-basedtreatment. Onlylegalbarriers and, in someplaces, reluctancestill stand in the way.

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Keywords: cannabinoid, plant, endocannabinoid, brain

PATOFYZIOLOGIE KANABINOIDŮ A JEJICH KLINICKÉ POUŽITÍ

prof. MUDr. Richard Rokyta, DrSc.
Konopí a věda X, Brno, 23. 4. 2025

ÚVOD

Kanabinoidy, hlavní účinné látky obsažené v konopí, se v medicíně využívají po tisíciletí. Podobně jako v případě opioidního systému byl v lidském těle objeven i endokanabinoidní systém, který hraje klíčovou roli při ovlivňování bolesti a dalších fyziologických procesů. Kanabinoidy prokazatelně působí na neuropatickou a nádorovou bolest, přičemž se uplatňují mechanismy jako neuroplasticita, fenomén wind-up nebo hyperexcitabilita. Významné terapeutické účinky byly zaznamenány také u pacientů s roztroušenou sklerózou, kde kanabinoidy zmírňují spasticitu a bolest, přispívají ke kvalitnějšímu spánku a zlepšují chuť k jídlu.

PATOFYZIOLOGIEKANABINOIDŮ

Nejznámější účinnou látkou konopí je delta-9-tetrahydrokanabinol (THC), který má jak psychoaktivní, tak léčebný potenciál. Další klíčovou sloučeninou je kanabidiol (CBD), který rovněž vykazuje terapeutické účinky, avšak bez psychotropních vlastností. Bylo prokázáno, že CBD může dokonce potlačovat některé negativní účinky THC, například úzkost nebo paranoia. Kromě těchto dvou látek se v konopí nachází i další kanabinoidy, například kanabigerol (CBG) s protizánětlivými účinky či tetrahydrokanabivarin (THCV), který se zkoumá v souvislosti s léčbou epilepsie a Parkinsonovy choroby.

Kanabinoidy působí primárně prostřednictvím CB1 a CB2 receptorů. CB1 receptory se nacházejí především v mozku a ovlivňují psychické a kognitivní procesy, zatímco CB2 receptory jsou součástí imunitního systému a podílejí se na zánětlivých procesech. Přestože konopí vykazuje široké spektrum pozitivních účinků, jeho použití může být spojeno s určitými riziky. Mezi ně patří možné psychiatrické poruchy (například úzkost, deprese či psychóza u predisponovaných jedinců), kardiovaskulární problémy (tachykardie, ortostatická hypotenze) nebo negativní vliv na hormonální systém a plodnost.

KLINICKÁ PRAXE

V klinické praxi se kanabinoidy osvědčily především v léčbě chronické bolesti, kde mohou být stejně účinné jako opioidy, ale s nižším rizikem závislosti a vedlejších účinků. Zejména u neuropatické bolesti se ukázalo, že kanabinoidy mohou snižovat bolestivost a zlepšovat kvalitu života pacientů. Pozitivní účinky byly rovněž zaznamenány u pacientů s roztroušenou sklerózou, kde konopí zmírňuje spasticitu, bolest a problémy s močovým měchýřem. Další potenciální oblasti využití zahrnují Touretteův syndrom, kde kanabinoidy pomáhají tlumit motorické i zvukové tiky, nebo glaukom, kde dokážou krátkodobě snižovat nitrooční tlak.

OPIOIDY A KONOPÍ

Výzkumy ukazují, že kanabinoidy mohou snižovat potřebu opioidů u pacientů s chronickou bolestí, což může vést ke snížení jejich toxicity a nežádoucích účinků. Některé studie dokonce naznačují, že léčebné konopí může být v terapii bolesti stejně účinné jako opioidy, přičemž přináší i širší spektrum výhod, například lepší spánek a emoční pohodu.

ZÁVĚR

Přestože léčebné konopí vykazuje velký terapeutický potenciál, jeho rekreační užívání není z lékařského hlediska vhodné. U dospívajících může negativně ovlivnit vývoj mozku, způsobit kardiovaskulární potíže a zvyšovat riziko vzniku závislosti. Z tohoto důvodu by léčebné užívání konopí mělo být pečlivě monitorováno a dávkování přizpůsobeno individuálním potřebám pacienta.

PROGRESS IN CULTIVATION SCIENCE FOR ENHANCED SECONDARY METABOLITES AND YIELD IN CANNABIS

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INTRODUCTION

The medicalpotential of cannabis (Cannabissativa L.) isbased on the complexchemical profile, comprisinghundreds of secondarymetabolitesincludingcannabinoids, terpenoids and flavonoids. Cultivation and environmentalconditionsweredemonstrated to affect plant development and function, and production of secondarymetabolites in cannabis. The increasingdemand for high-gradecannabis products stress the need for science-basedknowledge of cannabis plant-science, as understanding the regulation of plant responses to cultivationconditionsiskey for developing optimalchemical profile for modern medicine.

In the talk, we willdiscussourrecentresultsconcerning the potential of a range of cultivationconditions to regulate plant development and the profile of activesecondarymetabolites in 'drug-type' cannabis.

MATERIALS AND METHODS

In a range of experiments, we studied the effects of a cultivation conditions including the supply of mineral nutrients (N, P, K, Mg, Ca, Zn, Mn), light quality and intensity, plantarchitecturemanipulation, plantingdensity, pest management treatments, imposition of stress conditions prior to harvest and more, on plant development, function, yield quantity and quality. The plants were cultivated in pots under controlled conditions in-door, or in a greenhouse, and all experiments were conducted with 5–10 independent replicates.

RESULTS

Ourresultsrevealedsensitivity of the secondarymetabolite profile in medicalcannabis to a range of cultivationconditions, includingmineralnutrition [1], lightquality [2] and intensity, plant architecturemanipulation (structure and pruning) [3], temperature, plantingdensity, pest management treatments, and more.

CONCLUSION

The sensitivity of the chemical profile to cultivationconditionsstresses the importance of knowledge on the cannabis plant responses to cultivationschemes for the optimization of cultivation for production of high-qualitystandardizedmaterial for the medical market, as well as for development of plant products containingspecificdesirablechemicalprofiles.

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Keywords: Cannabis; cannabinoids; nutrition; plant-architecture.

GLOBAL REGULATORY AND COMMERCIAL DEVELOPMENTS IN CANNABIS FOR MEDICINAL PURPOSES

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SUMMARY

Cannabis has medical value, but it is not a medicine by default.

The 2022 UN rescheduling of cannabis and its resin from Schedule 4 to Schedule 1 acknowledges cannabis's therapeutic potential. Despitethis, onlya minority of doctorsarewilling to prescribedue to the paucity of properclinicalevidence and availability of cannabismedicines. In thisvacuum, recreationalcannabisisoftenpresented as a 'medicine' to patients.

The UN rescheduling now eases the legal supply of ‚cannabis materials and medicines' without violating UN Conventions. However, the rapid global expansion of the cannabis industry in the last decade has led to misunderstandings and misinterpretations of the regulations governing cultivation, manufacture, and distribution. Regulations, policies, and standards vary widely across the globe, resulting in inconsistent quality control.

In contrast, opioid medicines derived from the opium poppy have advanced rapidly since the 1930s. Their widespread use has driven improvements in product quality, generated clinical knowledge, and provided insights into the safety and efficacy of opioid medicines. Cannabis medicines lag behind due to decades of prohibition and a lack of scientific and clinical research.A comprehensive global approach to bridge this gap is urgently needed. Global alignment of regulations, standards, and practices is crucial. This alignment will enable regulators, standards organisations, industry, laboratories and the health profession to ensure a safe and consistent supply of quality cannabis materials and medicines.

The primary challengeis in standardising a botanicalstartingmaterial – the flowers of the cannabis plant. Since the cannabis flower is the building block of cannabis medicines, standardisation begins at cultivation.Consistent cultivation practices ensure uniform batch-to-batch cannabinoid content (w/w) and a reproducible chemical fingerprint. This consistency is essential for managing industrial contracts, meeting manufacturing input needs, and fulfilling contractual agreements requiring supplier verification and audit.

Variousmonographs on Cannabissativa L. inflorescenceareproposedorpublished in officialpharmacopeia. These compliance-oriented, quality-focused monographs enhance regulators' ability to audit, monitor, and detect issues within laboratories and the industry.

Developing robust, effective methods adopted by the industry is necessary. These methods must be cost-effective to ensure countries and organisations with limited resources can comply.

PSYCHOMODULATORY SUBSTANCES: NEW REGULATION OF PSYCHOACTIVE SUBSTANCES IN CZECHIA

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INTRODUCTION

Prohibition has been the globally dominant concept for the regulation and control of psychoactive substances for almost 70 years. It is based on the unrealistic ideology that the use of psychoactive substances other than in a medical context should be prohibited.Its effectiveness and legitimacy has been repeatedly questioned.

MATERIALS AND METHODS

Review of the background, principles and the main elements of the new regulatory framework alternative to prohibition will be presented.

RESULTS

Punitive prohibition violates human rights, decreases weel-being, increases health and societal harms. It also hampers the implementation of alternative policies and thus maintain the gap between non-regulation and prohibition. To bridge this gap (i.e. dilemma between non-regulation and drug prohibition), the new regulatory frameworkfor psychoactive substances with acceptable risks to be marketed for human non-medical consumption hasbeen adopted in Czechia (called PsychomdulatorySubstances). Safety profile of the substance, packing, information to consumers, health and safety warnings, restrictions in marketing or accessibility for minors will be regulated. Kratom and low-THC cannabis are the first substances considered for the new regulatory framework.

CONCLUSION

The regulation of Psychomodulatory Substances represents a shift towards modern policy reducing harms associated with psychoactive substances.

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Keywords: prohibition; regulation;psychomodulatory substance; low-THC cannabis

THE IMPORTANCE AND THERAPEUTIC POTENTIAL OF CBD IN VETERINARY CARE

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The healing potential of cannabis, backed by scientific evidence from modern research, has earned its place in human medicine over the past few years. Cannabis is prescribed for defined indications in accordance with current legislation, and there have been significant advances in the dosage forms and routes of administration used (capsules, vaporisation, ointments). It is therefore not surprising that, in the light of these positive developments in the use of medicinal cannabis in humans, there is growing interest in the possibility of its use in veterinary medicine, particularly among owners of so-called companion animals (dogs, cats) and horses. In other words, human medicine is an inspiration for the use of cannabis for veterinary purposes and owners are starting to ask questions, perhaps because they use or know cannabis themselves.

The structure of the endocannabinoid system is the same in humans and animals. Interestingly, however, the response to some cannabinoids in animals was sometimes different from that in humans. In animals, it is certainly an advantage that pre-clinical testing has often already been carried out on some specific species and in some cases, these could be the target species. In larger mammals, however, the data are unfortunately already very limited. Another problematic factor to take into account is that the cannabis plant contains THC with psychotropic effects. These could affect the animal‘s behaviour (for example, its movement) and the development of aggressive behaviour cannot yet be completely ruled out.

The situation is very different when it comes to the possible use of isolated cannabinoids, in particular cannabidiol (CBD). Therefore, our presentation summarises the potential therapeutic implications of CBD within clinical background, particularly in dogs and horses in the context of pain management, epilepsy and anxiety, with the aim of providing veterinarians with a brief overview of the up-to-date scientific knowledge in this field.

Keywords: Cannabinoids; Cannabidiol; Veterinary Medicine.

LEGISLATIVE REQUIREMENTS FOR THE USE OF CANNABIS PLANT AND EXTRACTS IN VETERINARY CARE (part I and II)

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If we follow the definition given by the current legislation, a veterinary medicinal product (VMP) is any substance or combination of substances that meets at least one of the following conditions: (a) it is presented as having properties for treating or preventing disease in animals; (b) its purpose is to be used in, or administered to, animals with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action ((c) and (d) do not appear to be cases specifically relevant to the area under consideration), then the products containing cannabis plant or extracts claimed to have therapeutic effects should be considered veterinary medicinal products and should therefore comply with the rules laid down in Regulation (EU) 2019/6 and the Act on Pharmaceuticals no. 378/2007, Coll.In addition, in the veterinary care sector, we must also comply with the legislative rules on the handling of the psychotropic substances.

Products other than VMPs (or feed or biocidal products) may be approved as veterinary (non-medicinal) products in accordance with Act No. 166/1999, Coll. on Veterinary Care. Veterinary products are defined in the Act as mass-produced products intended for animals, in particular dietetic, vitamin, mineral and cosmetic products, or for special laboratory diagnosis. For cannabis plants and extracts, there is also a specific guideline describing the conditions to be met by the applicant for approval of those veterinary (non-medicinal) products.

Our presentations will provide a brief overview of the current situation in the use of cannabis products in veterinary care with respect and emphasis on the requirements related to the current legislative framework.

Keywords: Legislation; Veterinary Medicinal Product; Veterinary (Non-medicinal) Products; Cannabis Plant and Extracts.

NOVEL APPROACH IN ANALYSIS OF PHYTO- AND ENDOCANNABINOIDS IN PLANT AND BIOLOGICAL SAMPLES UNDER IN VIVO CONDITIONS

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INTRODUCTION

Medicinal cannabis is gaining increasing interest in the scientific world due to pharmacological properties ofphytocannabinoids (PCs). In nature, PCs are found in less pharmacologically active forms, i.e. acidic forms, which are converted to neutral, pharmacologically active forms through decarboxylation. Neutral PCs affect endocannabinoid system, and influence the activity of endocannabinoids (ECs)in humans [1]. This study aim to in vivo monitoring of the profile of particular PCs in different Cannabis spp. cultivated under controlled conditions, and the analysis of the impact of medicinal cannabis on the level of ECs in blood and brain of rats.

MATERIALS AND METHODS

In this project solid-phase microextraction (SPME) along with instrumental analysis (liquid chromatography mass spectrometry, LC-MS) will be used. Biocompatible SPME probes were applied for in vivomonitoring the composition of different major and minor PCs in CBG-, and CBD-dominant cannabis plants. SPME sampling was implemented for profiling of selected ECs in blood and brain tissues of rats.

RESULTS

The developed method facilitated the successful extraction (in vivo SPME), separation and analysis (LC-MS) of multiple PCs in various medicinal cannabis plants, and also for monitoring trace levels of ECs, namely AEA, NADA, and 2-AG incomplex biological samples.

CONCLUSION

The proposed methodology based on SPME sampling along with advanced instrumental analysis facilitates precise analysis of PCs at different stages of plant growth, and also concomitant analysis of PCs and ECs in biological samples.

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Keywords: drug analysis; phytocannabinnoids; endocannabidoides; solid-phase microextraction;

PERSPECTIVES OF CANNABIS-BASEDMEDICINES IN HUMANS.
OPTIMIZATION OF SPME-LC-MS/MS METHOD FOR ANALYSIS OF SELECTEDENDO-ANDPHYTOCANNABINOIDS IN SERUM SAMPLES.

Magdalena Kaszewska[1], Katarzyna Woźniczka[1], Katarzyna Sztormowska-Achranowicz[2], Václav Trojan[3, 4], Agnieszka Mosińska[1], Anna Roszkowska[1]

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INTRODUCTION

The pharmacokinetics of phytocannabinoids (PCs), includingdelta-9-tetrahydrocannabinol (Δ9-THC) andcannabidiol(CBD) in humans are not fully understood, and data from conducted clinical studies are inconsistent. There are many variables that affect the pharmacokinetics of these compounds in the body, including health status, body fat content, diet, patient genetics, route of drug administration, and interactions with other medications.The endocannabinoid system is a complex neuromodulatory system thataffects the functioning of the central and peripheralnervous system in the organism [1]. Ligands of cannabinoidreceptors, in addition to endocannabinoids (ECs), mayalso be PCs obtained from plants of Cannabisspp. species [2]. The functioning of this system and the impact of PCs arestill not fullyunderstood.

MATERIALS AND METHODS

In thisstudy, solid-phasemicroextraction (SPME) was applied for isolation of ECs and PCs along with theirmetabolites from serum samples.For preciseanalysis of thosecompounds, advancedmethodbased on liquidchromatographycoupled to tandem mass spectrometry(LC-MS/MS) wasused.

RESULTS

Extraction of testedcompounds was carried out for 30 min. The analyzedcompoundswereseparated on an ACE Excel C18-AR chromatographiccolumn. The analysiswereperformed in positiveionmode.The optimized SPME-LC-MS/MS methodenabledsimultaneousisolation and analysis of ECs, and PCs along with theirmetabolites, and alsointernalstandardswithin 6 min.

CONCLUSION

SPME-LC-MS/MS method may enable future monitoring the levels of ECs, PCs and their metabolites in real biological samples, as well as the pharmacokinetics ofΔ9-THC and CBD.

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WHERE ARE YOU HEADING TO, CANNABIS RESEARCH

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INTRODUCTION

Cannabis is one of the most widely grown multi-purpose crops. It has been at the center of scientific attention for many years, mainly due to its unique chemical composition. The strongest natural fibers possessing antibacterial properties can be obtained from plant stalks. Seeds contain oil with well-balanced fatty acids and seed meal is a good source of digestible protein. In addition, Cannabis plants produce secondary metabolites, including bioactive cannabinoids, terpenes, phenolic compounds, and alkaloids. Phytocannabinoids, predominantly produced in Cannabis, represent thoroughly studied class of the compounds with wide range of pharmacological activities. In addition, more than 40 jurisdictions have undertaken policy reforms to liberalize the use of Cannabis products for medical and/or non-medical adult use.

MATERIALS AND METHODS

N/A

RESULTS

10 years of Cannabis research performed by scientists of Palacky University and Crop Research Institute (transformed to Czech Agrifood Research Center in 2025) is summarized in the presentation. It covers variety of research areas including analytical chemistry, plant sciences, agronomy, food science and pharmacology. Another aspect of research – collaboration with relevant partners such as St. Anna‘s University Hospital, AgritecŠumperk, Mendel University in Brno and Czech University of Life Sciences Prague will be discussed.

CONCLUSION

It is also crucial to secure microbial, chemical quality and safety of Cannabis products, likely via introducing international standards based on interlaboratory validation. Finally, technical and ethical challenges associated with promising tools to improve the quality and yield of Cannabis production, such as in vitro-, new breeding techniques and polyploidy should be solved.

BIOAFFINITY-BASED CONCEPT FOR METABOLITE PANEL CHARACTERIZATION

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INTRODUCTION

Rapid drug and alcohol detection techniques have become a growing necessity for the safety of law enforcement, first responders, and the general public. Current analysis of drug consumption requires invasive blood or urine samplest hat are not field applicable and laboratory costs for these tests are considerably higher compared to a non invasive approach. Thereis no field-deployable detection kit for delta 9-tetrahydrocannabinol (Δ9-THC), the psychoactive component of marijuana, due to lipophilicproperties of the parentcompound and metabolites, whichleads to prolongedstoragewithin the body and falsepositive test resultsregardingintoxication from THC. A roadsidealcoholdetection system thatisquantifiable and eliminates the need for laboratory analysisis also necessary as breathalyzers are typically used in combination with blood alcohol concentration values.

MATERIALS & METHODS

The concept for THC detection was established via immunoassayusing the following primary components: (±)-11-nor-9-carboxy-Δ9-THC (THC-COOH), THC antibody (monoclonalmouse),and THC-horseradishperoxidase (HRP; E.C. 1.11.1.7), hydrogenperoxide, and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The HRP assay was analyzedat 405 nm with a UV-vis spectrophotometer.
The concept for ethanoldetection was developedusing a modified HRP assay with 200 proof-absolute, anhydrousethylalcohol.

RESULTS

This research has shown that the THC metabolite can be quantified at nano molar levels based on the antibody-antigeninteraction from the HRP assay, which can be immobilized on a field-deployable polystyrene strip. Results from the alcohol quantification system also show the potential for component immobilization on a field deployablestripsuch as those which can be developed for THC with as little as 3 μL of sweat. This alcohol system accurately provided colorimetric feedback between 0.0-0.08% similar to a breathalyzer and human subject concentrations were accurately determined.

CONCLUSION

This result reporting provides anopportunity to integrate these systems into field deploy able applications with handheld UV-visible spectrophotometry devices and smartp hones that have specialized software for colorimetric analysis presented with a built-in camera. This technology can pave the way for on-site visual tests of drugs and alcohol and canallow law enforcement and first responders to operate in the moment of need rather than submitting samples from the individual involved who has to travel to a facility and provide blood or urine samples for laboratory analysis.

Key Words: Biosensors, Criminalistics, Forensics

MEDICAL CANNABIS ADD - ON THERAPY IN PATIENTS WITH TRIGEMINAL NEURALGIA

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INTRODUCTION

Neuropathic pain belongs to the most difficult pain conditions in patients to treat. Especially in patients with multiple sclerosis due to demyelinating plaques. Prevalence is between 1,9 % and 6,3 % in patients with multiple sclerosis [1]. The first-line therapy for trigeminal neuralgia secondary to multiple sclerosis are centrally-acting drugs: carbamazepine, lamotrigine, gabapentin, pregabaline. Some of them are relatively poor tolerated because of the side effects or they are insufficient in monotherapy. There is a need for combined therapy for the pain management and better efficacy of the treatment to improve the quality of life in these patients. Surgical procedures include Gasserian ganglion percutaneous techniques, gamma knife radiosurgery and microvascular decompression.

MATERIALS AND METHODS

It presents a clinical case report of a 48-year-old female with trigeminal neuralgia in distribution of the third branch of trigeminal nerve as a first manifestation of multiple sclerosis. The diagnosis was verified on MRI with angiogram. There were findings of a large amount of demyelinating plaques supratentorial and infratentorial include entry zone of the trigeminal nerve (some of them were active) and no signs of neurovascular conflict. The result of the cerebrospinal fluid analysis supported the diagnosis of MS. The patient was treated by pulse of corticosteroids followed by biological therapy with ozanimod. At the beggining the patient did not have benefit from gabapentine in monotherapy. So we switched the therapy gradually to combination of pregabaline 600 mg daily, carbamazepine 800 mg daily and as add-on therapy with medical cannabis (0,0625 g daily). As necessary tramadol/paracetamol 75/650 mg.

Patient refers improvement in mood, pain fluctuates but it is tolerable.

CONCLUSION

The medical cannabis is an option for the add-on therapy in constant, intolerable, chronic neuropathic pain as well for patients with trigeminal neuralgia as polyneuropathy.

The drug prescription needs to be tailored to each patient. We can take the advantage of the sodium – channel blockers (carbamazepine, oxcarbazepine), Voltage-Gated Calcium Channel (prebagaline) [2] and medical cannabis for the pharmacological treatment of trigeminal neuralgia.

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Keywords: medical cannabis, sodium-channel blockers, voltage-gated calcium channel, multiple sclerosis

Posters

EFFECTS OF PHYTOCANNABINOIDS ON NRF2 TRANSLOCATION IN HUMAN SKIN KERATINOCYTES

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INTRODUCTION

Cannabis sativa preparations are very popular in dermal use for their analgesic and anti-inflammatory properties. Phytocannabinoids (PCBs) are responsible for *C. sativa* biological activity. The main biologically active and most studied PCBs are non-psychotropic cannabidiol (CBD) and psychotropic (-)-Δ9-trans-tetrahydrocannabinol (THC) [1]. Nrf2 protein is a redox sensitive transcription factor that drives expression of many cytoprotective and keratinocyte epidermal differentiation genes. Electrophiles stimulate Nrf2 translocation to nucleus, where it controls expression of genes [2].

The aim this study was to compare ability of CBD, most studied PCB,with other selected PCBs cannabigerol (CBG), cannabichromene (CBC),THC, cannabinol (CBN, formed from THC during processing)to modulatethe Nrf2 pathway in normal human epidermal keratinocytes (NHEK). An endocannabinoid anandamide (AEA) was also studied.

MATERIALS AND METHODS

NHEK were seeded on microscope slides or Petri dishes in EpiLife medium with growth supplement kit (HKGS). Then cells were treated with CBC, CBD, CBG, THC, CBN or AEA(1 μM) and sulforaphane (SFN, 1 μM), aknown Nrf2 stimulator,in the medium without HKGS for 6 h. Then cells were fixed and Nrf2 protein was detected immunocytochemically (brown colour). The nuclei were stained with hematoxylin (blue colour). Cells were analysed under light microscope (magnification 200×) and results (microphotographs) were processed in ImageJ software. Effect of cannabinoids (1 μM) on Nrf2 protein level was evaluated after 24 and 48 h incubation by western blot.

RESULTS

CBC, CBD and CBG stimulate Nrf2 nuclear translocation more than CBN and THC. AEA had a similar effect as the positive control SFN. CBD showed the highest effect on Nrf2 translocation of all studied compounds. All PCBs increased Nrf2 protein after 24 h, but only CBD elevated Nrf2 protein level after 48 h.

CONCLUSION

All tested PCBsmodulate the Nrf2 signalling pathway and thus they could modulate keratinocyte differentiation and skin barrier function with long-term dermal application.

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Keywords: Phytocannabinoids; *Cannabis sativa*; Nrf2 translocation; Human keratinocytes

SAFETY ASSESSMENT ON CBD-RICH HEMP EXTRACT IN SUB-CHRONIC CROSS-SEX STUDY WITH RATS

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Cannabidiol (CBD) is a phytocannabinoid of Cannabis sativa L., and is currently increasingly widely used for medical purposes. Here we focus on the safety and pharmacokinetics of CBD-rich full-spectrum hemp extracts (77% w/w) in male and female rats. Subchronic toxicity testing for 90 days was conducted using doses of 0.5, 5, 10, and 35 mg CBD extracts/kg/day orogastrically administered. No adverse effects and disturbances in organs or body weight, behaviour, locomotion, food intake or morbidity/mortality were observed. The pathomorphological examination did not show any changes in the gastrointestinal tract and liver. Blood cell analyses showed a significant difference between control and the treated animals in leukocyte, mean corpuscular haemoglobin concentration, mean corpuscular volume of erythrocytes, and number of neutrophils and monocytes. However, blood cell analysis showed significant sex-dependent differences, such as haemoglobin and erythrocyte count. In the treated animals, the changes of levels of ions (Ca^{2+}, Na^{+}, K^{+}, Cl^{-}), alkaline phosphatase, and creatinine were also observed in both sexes. Males showed decreased alanine transaminase levelsand females showed hyperalbuminemia. CBD was quantified in treated animals in dose dependent manner. The accumulation of CBD in individual tissues increased in the following order: brain, serum, liver, heart, kidneys, muscles, and skin. The results showed sex-dependent latent disruption of kidney and liver homeostasis, most probably reversible in nature.

IN VITRO CYTOTOXIC ACTIVITY OF SOLANINE ON SELECTED CANCER CELL LINES

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INTRODUCTION

Solanine is a glycoalkaloid found in plants of the Solanaceaefamily.The highest amount of solanine can be found in green parts of tubers, sprouts, and leaves of potatoes *(Solanum tuberosum)*,which originate from South America and now are cultivated worldwide [1]. Solanine is known for its anti-pyretic,anti-inflammatory, and antibiotic activity [2]. Recently, attention has been drawn to its potential anticancer properties, and the initial results have been promising [3].In our study, we evaluated in vitro cytotoxicity properties of solanine. The cytotoxicity was estimated on human cancer cell lines such as gastric cancer – AGS, melanoma – A-375 and cervical cancer – HeLa, and non-cancer human fibroblasts.

MATERIALS AND METHODS

Solanineobtained from Phytolab (Germany)was dissolved in DMSO at the concentration of 20mg/mL.

The human gastric adenocarcinoma AGS, cervical cancer HeLa, and melanoma A-375 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Fibroblasts were obtained from LGC Standards (Germany).AGS cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM)/Ham's F-12. HeLa, A-375 cells and fibroblasts were maintained in Dulbecco's Modified Eagle's Medium (DMEM) with low glucose and high glucose, respectively. All media were supplemented with 100 units/mL of penicillin, 100 µg/mL of streptomycin, and 10% (v/v) fetal bovine serum (FBS) (Merck Millipore, Burlington, MA, USA). The cells were incubated at 37 °C with 5% CO_2.

To estimate the cytotoxic effect of solanine, an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was performed. All cell lines were seeded in 96-well plates at a density of 5×10^3 cells/well and treated for 24 hours with the plant metabolite at concentrations of 1.5–22.5 µg/mL for AGS and HeLa, and 0.5–10 µg/mL for A-375. The DMSO concentration did not exceed 1% (v/v) for all the cell lines.The data are expressed as percentage of viability and also as IC_{50} values (µg/mL). Oxaliplatin was used as a positive control.

RESULTS

Solanine exhibited cytotoxic activity against all tested cell lines, with the strongest effect observed in melanoma A-375 cells, which showed an IC_{50} value of 3.41 ± 0.20 µg/mL.In the case of gastric cancer AGS cells and cervical cancer HeLa cells, the IC_{50} values were 6.30 ± 0.35 µg/mL and 6.73 ± 0.64 µg/mL, respectively.The viability of A-375 cells decreased from 86 ± 5% at a concentration of 1 µg/mL to 19 ± 3% at 10 µg/mL. In contrast, AGS and HeLa cell viability decreased from 78 ± 6% to 22 ± 2% and from 81 ± 1% to 23 ± 2%, respectively, over the concentration range of 3 to 22.5 µg/mL.

CONCLUSION

Results of our experiment demonstrate that solanine possesses significant cytotoxic activity against human cancer cell lines in vitro. The strongest effect was observed in melanoma A-375 cells, suggesting a higher sensitivity of this cell type to the compound. While AGS and HeLa cell lines also responded to solanine treatment, their IC_{50} values were moderately higher. These findings support the potential of solanine as a candidate for further investigation. Additional studies including mechanism of action and in vivo activity are necessary to fully evaluate its therapeutic potential and safety profile.

Keywords: solanine; cytotoxic effect; cancer cells; Solanaceae

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MONITORING OF THE LEVEL OF ENDOGENOUS CANNABINOIDS IN THE HEART WITH THE USE OF SPME-LC-MS/MS METHOD

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INTRODUCTION

The endocannabinoid system (ECS) is a network of lipid signalingmolecules, theirreceptors, and metabolizingenzymes, alsoactive in the heart [1]. Endocannabinoidssuch as anandamide (AEA), 2-arachidonoylglycerol (2-AG), and N-arachidonoyldopamine (NADA) arelocallyproduced in cardiomyocytes, the endothelium, and smoothmusclecells [2]. Under pathologicalconditionslikehypertensionorheartfailure, ECS activityintensifies, influencingvasculartone, oxidativestress, and inflammation [2]. The aim of thisstudy was to develop a sensitivemethod for determiningendocannabinoidlevels in hearttissueusing SPME-LC-MS/MS, to assesstheirdistribution in variousanatomical regions, and to comparelevels in humanexplantedhearts and rat hearts (WistarHanstrain).

MATERIALS AND METHODS

Solid-phase microextraction (SPME) was used for extraction, followed by liquid chromatography. Optimization included sorbent selection, extraction/desorption time, and sample pH. Different coatings were tested for efficiency and matrix compatibility. Extractions were performed on homogenized and non-homogenized rat heart tissue. Extraction conditions were adapted based on previous studies [3].

RESULTS

The method was applied to analyze endocannabinoids in 3-month-old male rat hearts using biocompatible C18 probes (4 and 10 mm). Ten-minute static extraction enabled effective compound collection without additional processing. The method showed good repeatability and stability. Different heart regions were sampled to assess potential variation in endocannabinoid distribution. Extractions were successful in both homogenized and intact tissue samples, and the probes retained performance even after autoclaving.

CONCLUSION

SPME showspotential for analyzing biological tissues in both research and clinical settings, with key advantages such as real-time applicability and suitability for monitoring endocannabinoids in heart regions.

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Keywords: Endocannabinoids; Heart; SPME; LC-MS/MS.

PROFILING OF PHYTOCANNABINOIDS IN CBD- AND CBG-DOMINANT CANNABIS PLANTS WITH TWO EXTRACTION APPROACHES ALONG WITH HPLC-UV ANALYSIS

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INTRODUCTION

The increasing value of the medical cannabis market observed in recent years is associated with growing competitiveness among its producers. In raw cannabis plant material, acidic forms of major and minor cannabinoids (phytocannabinoids (PCs)) dominate, such as cannabigerolic acid (CBGA), and cannabinolic acid (CBDA). Currently, a gold standard method described in Cannabis flos monograph of the European Pharmacopoeia comprises the analysis of acidic and neutral forms of PCs in Cannabis spp. inflorescences with the use of ethanolic extraction [1]. As a complementary technique for extraction of PCs from collected plant inflorescences, solid-phase microextraction (SPME) can be implemented [2,3].

METHODS AND MATERIALS

Both techniques were used for extraction of PCs from small amounts (100 mg) of homogenized samples of CBG- and CBD-dominant cannabis flowers. Traditional extraction technique required the use of 10 mL of ethanol for each sample.

SPME-based extraction utilized C18 extraction phase. HPLC-UV analysis of obtained extracts was performed in gradient elution mode. The separation of analytes was performed with the use of Acsentis Express C18 chromatographic column (15 × 4.6, 2.7 µm) with total analysis time of 12.5 min.

RESULTS

The obtained results reveal differences in the composition of PCs between both variants of cannabis plants. In CBD-dominant plant, CBDA and its neural form – cannbidiol (CBD) dominated, whereas in CBG-dominant plant, CBGA and its neutral form – cannabigerol (CBG) was detected. In addition, in CBD-dominat plants small levels of other PCs were detected, including cannabinol (CBN).

CONCLUSION

The obtained results were compared in the respect to the amount of extracted PCs, and also sensitivity and selectivity of those two different extraction approaches. SPME coupled to HPLC-UV can be used as quality control tool to monitor the content of particular PCs during post-harvest processing and storage of medicinal cannabis products.

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Keywords: Phytocannabinoids, SPME, Ethanolic extraction, HPLC-UV

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