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The safety of cannabinoids and cannabis-based products

Summary of Ph.D. thesis

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LIST OF PUBLICATIONS RELATED TO THE THESIS

1) Czégény Zs, Nagy G, Babinszki B, **Bajtel Á**, Sebestyén Z, Kiss T, Csupor-Löffler B, Tóth B, Csupor D: CBD, a precursor of THC in e-cigarettes, SCIENTIFIC REPORTS 11 : 1 Paper: 8951 , 6 p. (2021), doi: 10.1038/s41598-021-88389-z
   IF: 4.997 SJR indicator: D1
2) **Bajtel Á**, Kiss T, Csupor-Löffler B, Szendrei K, Csupor D: Cannabis: gyógyszer, élelmiszer vagy kábítószer? [Cannabis: medicine, food or illicit drug?], ORVOSI HETILAP 162 : 45 pp. 1808-1817. , 10 p. (2021), doi: 10.1556/650.2021.32211
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3) **Bajtel Á**, Kiss T, Tóth B, Kiss Sz, Hegyi P, Vörhendi N, Csupor-Löffler B, Gede N, Hohmann J, Csupor D: The Safety of Dronabinol and Nabilone: A Systematic Review and Meta-Analysis of Clinical Trials, PHARMACEUTICALS 15: 1 Paper: 100 , 15 p. (2022), doi: 10.3390/ph15010100
   IF: 4.6 SJR indicator: Q1
4) Vida R Gy, Strauss L V, **Bajtel Á**, Kiss T, Csupor D, Fittler A: Safety and risks of CBD oils purchased online: unveiling uncertain quality and vague health claims, FRONTIERS IN PHARMACOLOGY 14 Paper: 1273540 , 12 p. (2023), doi: , 10.3389/fphar.2023.1273540
   IF: 4.4 SJR indicator: Q1
5) **Á Bajtel**, A Fittler, R Berkecz, P Püski, D Csupor, T Kiss: Less than labelled, but more than safe: Quantification of CBD content in food-supplements and hemp seed oils using a validated UHPLC-UV method, SUBMITTED ON 16 August 2024 to journal HELIYON.

The cumulative impact factor of the publications related to the thesis: 14.704

1. INTRODUCTION

The importance of studying *Cannabis sativa* L. has long been a focus of many scientists, and even the history of hemp as a medicine dates back to centuries. The structure elucidation of the major and most abundant cannabinoids, Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) was a huge landmark in the field of cannabinoid research thanks to Raphael Mechoulam, who elucidated the structure of these compounds in 1960's. Today, the significance of cannabis is even greater due to the increasing popularity of CBD and other cannabinoids as food-supplement and medicines.

Cannabis sativa L. is known for its psychoactive effects, more commonly 'getting a high feeling', but some of its constituents, such as CBD, are not psychoactive. The toxicological and pharmacokinetic studies of cannabinoids have evolved in parallel by the application of the chemical components, but the results of the safety studies could not keep up with the success of the application of cannabinoids. The administration of CBD-containing or enriched oils and food-supplements has been a risk factor to consumers due to the diversity of CBD concentrations in the products. There are no comprehensive studies that would investigate the various CBD-containing products in Hungary, although the number of products on the market would indicate the performance of such studies.

Currently, several active ingredients of cannabis (CBD and THC) and the semisynthetic derivatives of THC (nabilone) are marketed as medicines, but various other products can also be found on the market such as food-supplements, wellness, and beauty products. The problem with CBD products is not only the chemical profile, but also the presence of impurities and other minor cannabinoids that could come from the manufacturing technologies. In this way, analytical investigation of such products is highly suggested. Additionally, the legal status of cannabis and CBD is questionable in the European Union (EU) and outside of the Community because of its pharmacological effects administered as food-supplements. Nowadays, in addition to its rational use, there is also a significant unprofessional use based on exaggerated expectations. This is in part due to the use of hemp anomalies in the legal regulation of cannabis. Furthermore, the fact that cannabinoids are active substances in antiepileptic medicines can lead people to the use of cannabis and CBD-containing products with hope for several therapeutic fields without any medical control.

Statistical analysis is necessary for all types of research. Meta-analyses are good tools for evaluating big data connected to a defined topic. The number of clinical trials conducted with CBD is limited and emphasizes different outcomes. Adverse events are crucial parts of

any authorization process because without a reliable safety profile, active ingredients cannot be accepted. Applying the proper search terms and searching in the appropriate databases, meta-analyses can be easily performed. Focusing on adequate outcomes, meta-analyses can be solid sources of evidence-based medicine. The long-term use of cannabinoids in evidence-based medicine is greatly influenced by research that can provide a more accurate picture of their risk-benefit profile.

Based on the current domestic legislation, including member states of EU and international conventions in force on hemp and its constituents, we receive a mixed opinion of the plant. There is a lack of unified regulation in the EU on the marketing and quality of CBD products. Thus, the popularity of CBD makes its way through advertisements, social media, manufacturers, and other untrusted sources, respectively. All these facts highlight the need for proper quality control of CBD-based products that require swiftly and easily applicable analytical methods to determine their CBD content. In 2022, the European Food Safety Authority (EFSA) published a statement on the safety of CBD as a novel food, determined various gaps and uncertainties and concluded that the safety of CBD as a novel food cannot currently be established.

The work presented in this thesis is part of a research project on cannabinoids at the Institute. The previous results of the research team have shown that even the study of cannabinoids, which have been relatively well researched in terms of bioactivity, can still hold surprises, and an analysis of the literature shows that there are more questions than answers in the field of cannabinoid research. In my work, I present investigations conducted using different methodologies, with the common feature that they all focus on promoting a more rational and safer use of cannabinoids.

2. AIMS OF THE STUDY

The aim of our work was to analyze the safety of two widely used cannabinoids, dronabinol and nabilone, to collect basic research data on the safety of CBD-containing e-cigarettes, and to conduct a chemical analysis of CBD-containing food-supplements. To this end, we aimed to:

- analyze the safety of dronabinol and nabilone in a meta-analysis based on data from clinical trials;
- investigate the effect of temperature on the composition of CBD pyrolysis products under different conditions;

- develop a reliable and robust method for the screening of commercially available CBD-containing products in Hungary;
- qualitatively and quantitatively describe the examined products.

3. MATERIALS AND METHODS

3.1. Meta-analysis of clinical data on the safety of dronabinol and nabilone

The meta-analysis was performed applying standards of Cochrane Methodology protocol and PRISMA guidelines. PICO (patients, intervention, comparison, outcome) format was applied: P: adult patients; I: dronabinol or nabilone; C: placebo; and O: frequency of adverse effects. The meta-analysis was reported according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement. The meta-analysis protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) a priori (registration number CRD42021240190).

The literature search was conducted until 21 February 2020, using the following search strategy: [dronabinol OR nabilone] for EMBASE; [("dronabinol"[MeSH Terms] [All Fields]) OR ("nabilone"[MeSH Terms] [All Fields])] for PubMed; [dronabinol OR nabilone] for Cochrane Central Register of Controlled Trials; and [TOPIC: (dronabinol OR nabilone). No publication date or publication status or language restrictions were applied. For transparency, the meta-analysis was based on publicly available data; neither the authors of the articles nor the manufacturers of the studied products were contacted for additional information.
All randomised, placebo-controlled trials (RCTs) evaluating the clinical effects of dronabinol or nabilone and reporting adverse effects were included. For each outcome, at least three clinical trials involving different patient populations were required to perform a statistical analysis.
The process of study selection, data extraction, risk of bias assessment, and statistical analysis was performed following PRISMA guidelines. The detailed methodology is described in paper **2** related to this thesis (i.e. Bajtel Á et al. PHARMACEUTICALS 15: 1 Paper: 100 , 15 p. (2022), doi: 10.3390/ph15010100).

3.2. Pyrolysis studies of cannabidiol (CBD) with Py-GC/MS

A 1 mg/mL CBD in methanol solution was examined. The experiments were carried out in a gas mixture of 9.34% (n/n) oxygen and 90.66% (n/n) nitrogen or in a helium atmosphere.

Py-GC/MS analyses were performed using a Pyroprobe 2000 (CDS Analytical, Oxford, PA, USA) pyrolizer equipped with a platinum heating coil and a quartz sample tube. The

experimental conditions of this analyzis are described in details in **paper 1** related to this thesis (Czégény Zs et al. SCIENTIFIC REPORTS 11 : 1 Paper: 8951 , 6 p. (2021), doi: 10.1038/s41598-021-88389-z).

3.3. Quantification of CBD content in food-supplements and hemp seed oils by UHPLC-UV methods

The CBD-content of 27 products purchased from Hungarian online sources were quantified using validated UHPLC-UV method. The products included CBD-enriched oils, gelatine capsules containing CBD oil, e-cigarette liquid, and hemp seed oils. The label of the analyzed samples was also evaluated. The identification of the source or the products i.e. full-spectrum, broad-spectrum, distillate, were determined.

The methods of label analysis, sample preparation, method validation, and quantification are published in **paper 4-5** related to this thesis (Bajtel Á. et al. FRONTIERS IN PHARMACOLOGY 14 Paper: 1273540 , 12 p. (2023), doi: , 10.3389/fphar.2023.1273540; Bajtel Á. et al. SUBMITTED ON 16 August 2024 to journal HELIYON).

4. RESULTS

4.1. Safety of dronabinol and nabilone

4.1.1. Literature search and study selection

Dronabinol and nabilone search terms were used for the literature search of EMBASE, PubMed, Web of Science databases and the Cochrane Central Register of Controlled Trials, and removing duplicates, the search yielded a total of 7859 potentially relevant reports. After screening the titles, abstracts, full-texts 7667 articles were excluded. The reasons for excluding articles were not clinical studies, not a placebo-controlled setting, etc.. 19 RCTs were considered to be appropriate for quantitative analysis, and 16 of these were included in the meta-analysis. Although three studies were considered for inclusion, the criterion of the minimal number of studies with the same outcome was not met in any of the outcomes reported; therefore, they were excluded from the meta-analysis.

4.1.2. Risk of bias assessment

In general, the methodical quality of the trials included in our final quantitative analysis was considered good, mainly with a low or unclear risk of bias (**Figure 1**). None of the studies showed a high risk of selection bias. In nine studies, random sequences or codes were generated by computer programs. Therefore, these studies were judged to have a low risk of selection bias. However, the remaining seven studies had unclear risk of selection bias, because the authors failed to describe the methods used for randomization in detail. Based on the blinding

of the personnel and participants, and making the interventions as identical as possible, nine studies were reckoned to have low risk of performance bias. In the remaining studies, it was not mentioned whether the intervention and the comparator were identical in size, shape, color, and odor. Moreover, the authors of four of these studies failed to describe precisely who exactly was blinded. Ten trials had a low risk of detection bias. In these studies, the evaluation of the outcomes was done in a properly blinded manner. However, six trials were judged to have an unclear risk of detection bias because blinding of the outcome assessment was not described in detail, and it was unclear whether the person responsible for the assessment was blinded or not. Almost all studies showed a low risk of attrition bias. However, in one trial more than half of the enrolled patients did not complete the study; therefore, this study was judged to have a high risk of attrition bias. Six studies showed a low risk of reporting bias. In four studies, not all the results were clearly indicated numerically; these studies were considered to have an unclear risk of reporting bias. We identified several flaws, for example, inconsistency between the methods and the results section, missing results or p values, in six studies; therefore, these studies were considered to have a high risk of reporting bias. Overall, all studies showed a low risk of other types of bias.

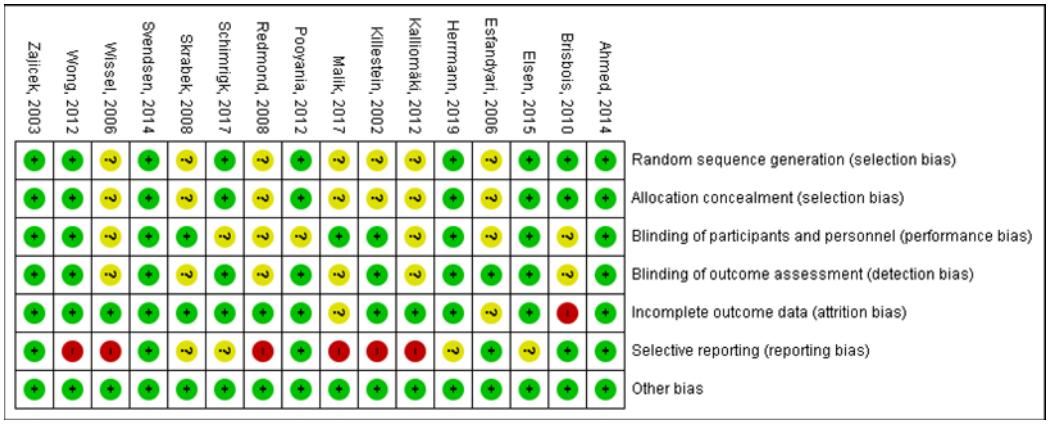

Figure 1. Table of biases

4.1.3. Study characteristics

Nabilone

In the case of nabilone, 5 of the 6 included trials used a crossover design. Clinical trials were carried out in Canada, the UK, and Austria/Germany/Switzerland. Nabilone was used to alleviate agitation in patients with moderate to severe Alzheimer's disease, spasticity in people with spinal cord injury, spasticity-related pain, fibromyalgia. In two trials, the effects of nabilone on capsaicin-induced pain and hyperalgesia, and the analgesic and antihypertensive

properties of nabilone on experimental heat pain were studied. The duration of these studies was 1 to 9 weeks. The patients were 18–70 years old (mean age 22.5–50.1 years), except in a trial in which patients with Alzheimer's disease were included and the mean age of the patients was 87 years. The applied dose ranged between 0.5–3 mg daily, in three trials 0.5–1 mg titration doses were used. Altogether, 154 patients were enrolled and 129 completed the studies.

Dronabinol

Dronabinol was studied in 10 randomized, placebo-controlled trials, conducted in Canada, Denmark, Germany, the Netherlands, the USA and the United Kingdom, and two of these trials were crossover. The duration of the study ranged from 2 days to 16 weeks. In the case of one study, dronabinol was administered 4 times, with wash-out periods of 2 weeks. 911 patients enrolled were 18–70 years old (mean/median age 26.0–72.1), and in some studies, only the mean age (46–79 years) was disclosed. Data from 774 patients were evaluated. Daily doses of dronabinol ranged between 2.5–15 mg. In two trials, the efficacy of dronabinol in the alleviation of neuropathic pain in patients with multiple sclerosis, and another trial focused on the efficacy and safety of the drug in patients with multiple sclerosis (MS). In one trial, the effect on gastrointestinal transit and postprandial satiation was studied in healthy human subjects, while in another trial the effect on gut transit was studied in patients with irritable bowel syndrome. One study aimed to determine if THC can improve taste and smell perception, appetite, caloric intake, and quality of life in cancer patients.

4.1.4. Outcomes

4.1.4.1. Quantitative analysis –nabilone

In studies evaluating the effects of nabilone, 39 different adverse effects were reported. These were classified according to the International Statistical Classification of Diseases and Related Health Problems (ICD-10) and divided into three main categories: AEs related to the central nervous system, cardiovascular system, and miscellaneous. 15 AEs were related to the central nervous system, while 5 affected the cardiovascular system. AEs were more frequent in the treated group than in the placebo group in both major types (68 vs 24 and 25 vs 6, respectively), and the same applies to the total number of AEs (228 vs. 61). Only 4 AEs (drowsiness, dizziness, headache, dry mouth) were reported in at least three studies and could be meta-analyzed. Drowsiness was more than seven times more frequent in patients treated with nabilone than in the placebo group (OR: 7.25; 95% CI: 1.64–31.95, **Figure 2A**), while the risk of dizziness (OR: 21.14; 95% CI: 2.92–152.75, **Figure 2B**) and dry mouth was also higher (OR:

0.94; 95% CI: 0.19–4.72, **Figure 2C**) in the nabilone group. However, the frequency of headache was not different in the two groups (OR: 17.23; 95% CI: 4.33–68.55, **Figure 2D**).

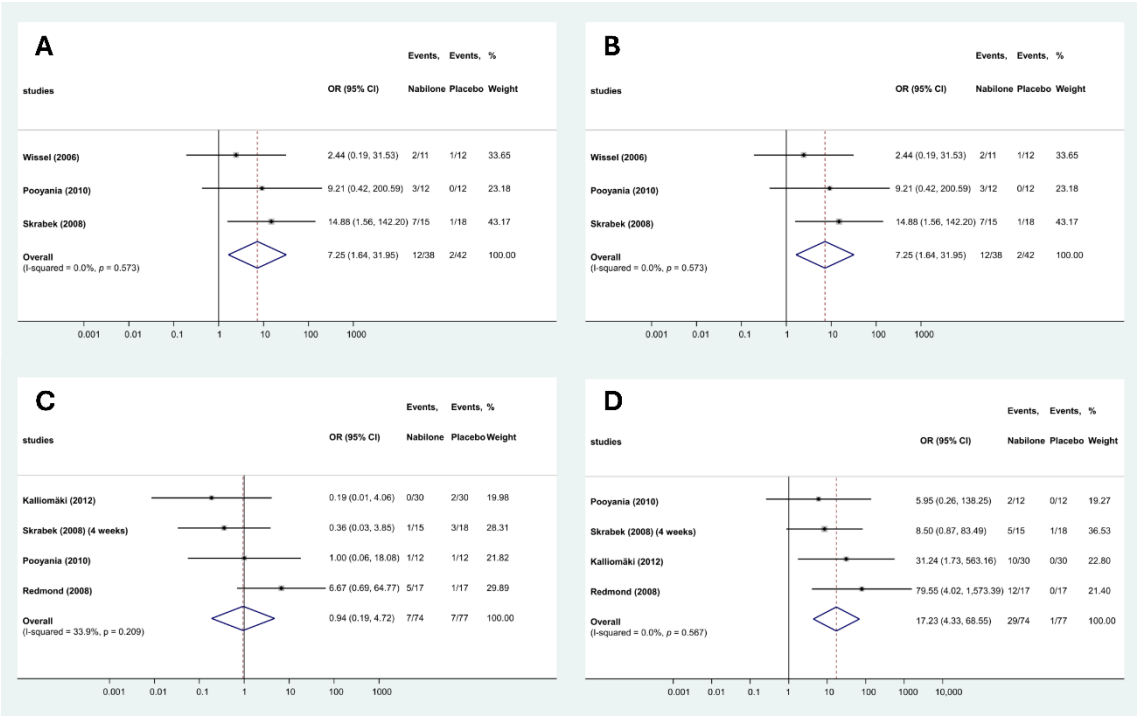

Figure 2. Forest plots of different AEs – nabilone

For robustness evaluation, a leave-one-out sensitivity analysis was performed for each AE. The summary ORs remained stable in the case of dry mouth and headache. However, in the case of dizziness and drowsiness, no significant differences can be observed for frequency AEs.

4.1.4.2. Quantitative analysis – dronabinol

In the analyzed clinical trials, 97 different AEs were reported. These were classified according to ICD-10 and grouped as AEs that affect the central nervous system, respiratory system, musculoskeletal system, gastrointestinal system, urogenital system, and miscellaneous. The frequency of AEs was higher in these domains in the dronabinol-treated groups (46 vs 11, 5 vs 2 and 17 vs 6, respectively) except for AEs related to the gastrointestinal and urogenital systems. The overall risk of adverse events was higher based on the total number of recorded events (325 vs 142). Altogether, 6 individual AEs (nausea, drowsiness, dizziness, headache, fatigue, dry mouth) met the criteria for the meta-analysis. The frequency of dry mouth (OR: 5.58; 95% CI: 3.19–9.78, **Figure 3A**), dizziness (OR: 4.60 95% CI: 2.39–8.83, **Figure 3B**) and headache (OR: 2.90; 95% CI: 1.07–7.85, **Figure 3C**) was significantly higher in the dronabinol groups, while in the case of nausea, drowsiness and fatigue there was no such difference [(OR:

1.45; 95% CI: 0.38–5.43, **Figure 3D**), (OR: 3.77; 95% CI: 0.43–33.25, **Figure 3E**), and (OR: 2.00; 95% CI: 0.82–4.88, **Figure 3F**), respectively].

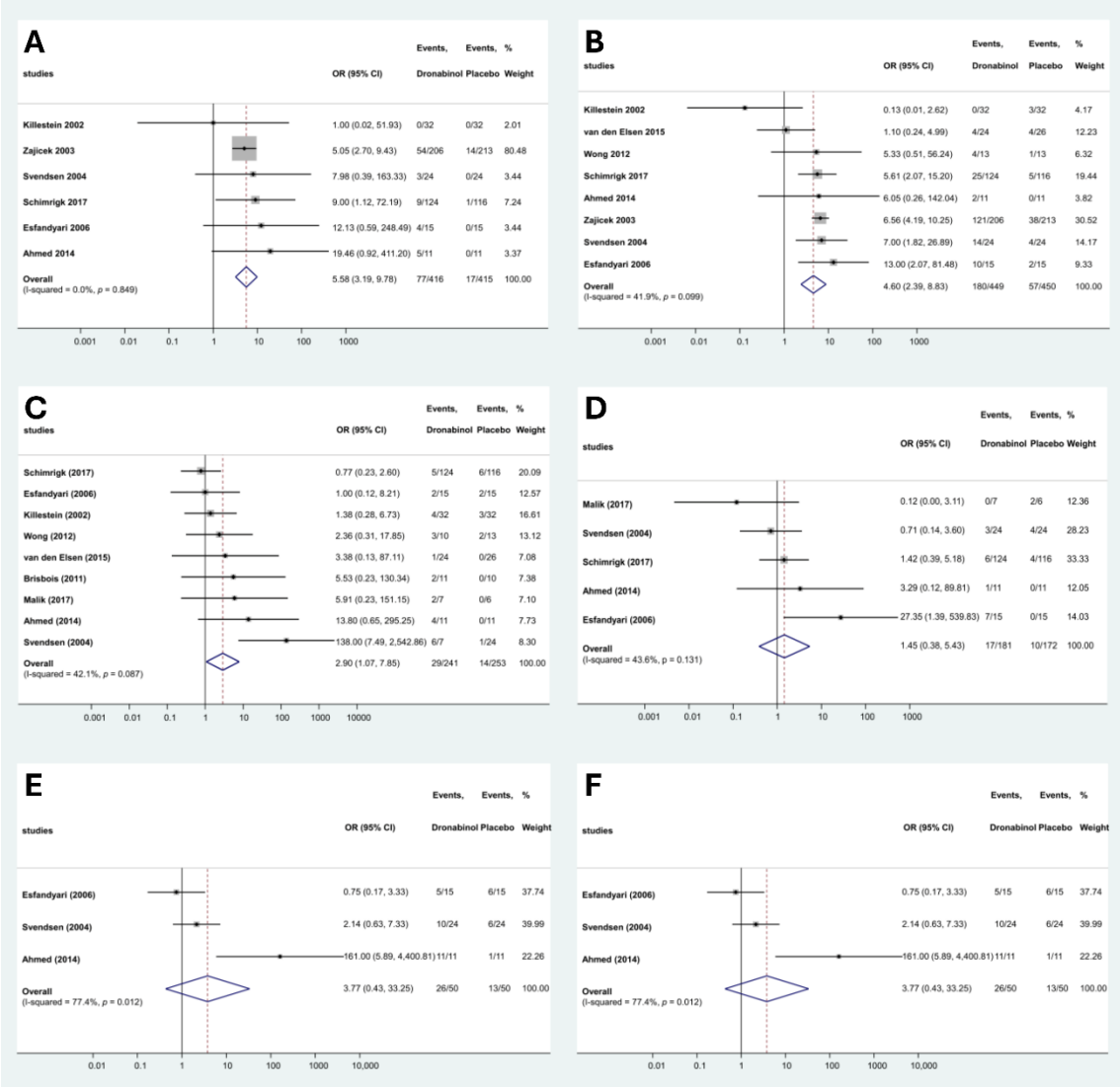

Figure 3. Forest plots of different AEs – dronabinol

Furthermore, sensitivity analyses by iteratively removing one study at a time showed similar and consistent results, indicating the robustness of our findings, except for headache, where in the case of removal of the results of other studies, the risk of AEs in groups treated with dronabinol or placebo was not significantly different.

4.1.5. Qualitative analysis of excluded studies

Although three randomized controlled studies were excluded from the meta-analysis, the results of these may also contribute to the overall picture of the AE profile of nabilone and dronabinol. One trial was left out because the number of studies reporting specific AEs was not sufficient

to prepare a meta-analysis, while one clinical trial was excluded due to inadequate reporting of AEs (using general terms instead of specifying AEs), and in one study, the numbers of different AEs were combined and could not be evaluated separately.

4.2. Transformation of CBD during pyrolysis

Depending on various factors the coil temperatures of e-cigarettes range from 110 to 1008 °C. In our experiments, we studied pyrolysis in the typical operating temperature range of e-cigarettes (250–500 °C) under both inert and oxidative conditions.

The composition of CBD pyrolyzates was obtained in inert and oxidative atmospheres. The data obtained clearly present the thermal instability of CBD. Depending on temperature and atmosphere, 25% to 52% of CBD transformed into other chemical substances during the experiments. In the absence of oxygen, 23 pyrolysis products were observed, 15 of which were identified. These compounds represented 81–27% of the pyrolysis products (at lower temperatures, between 250–400 °C, 93–97%). In the presence of oxygen, 22 pyrolysis products were detected, the 15 identified components represent the 88–96% of the degradation products in the studied temperature range. Most of the degradation products appeared in both inert and oxidative conditions. Additional four products were observed exclusively in the oxidative atmosphere, while five degradation products were observed exclusively in the inert atmosphere.

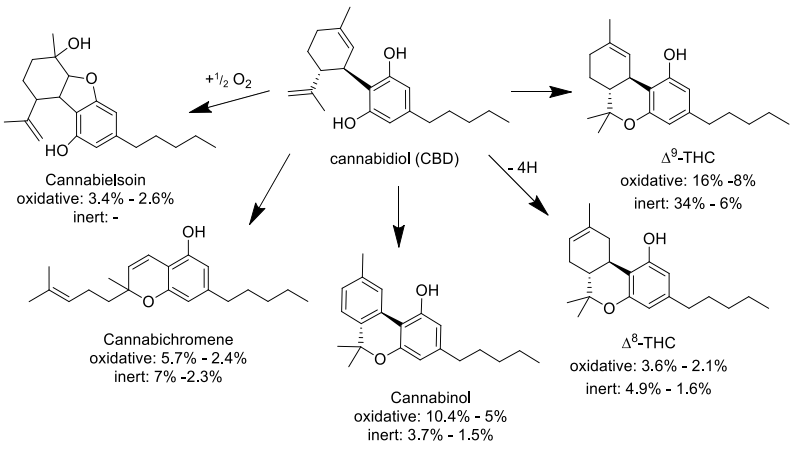

Figure 4. The major thermal decomposition routes of CBD

The four most intense products, namely Δ^9-THC, Δ^8-THC, cannabichromene and cannabinol, represent more than 95% of the decomposition products at pyrolysis temperatures of 250 and 300 °C in an inert atmosphere. Under oxidative conditions, an additional product, cannabielsoin, appeared. All of these compounds formed by the cyclization reaction (**Figure 4**). The cyclization of phenolic flavors to bicyclic compounds under simulated tobacco heating

conditions at 300 °C was previously reported. Analogously, in the present case, one of the phenolic O of CBD was linked to the tertiary carbon of the isopropenyl group, thus forming a sterically favored six-membered ring and the resulting Δ^9-THC molecule. However, Δ^9-THC formed also under inert atmosphere. The psychoactive Δ^9-THC was the main compound detected, accounting for up to 42% and 70% of the decomposition products under oxidative and inert conditions, respectively, at all temperatures applied. One possible reason for the lower Δ^9-THC amount in the oxidative atmosphere measured in our study could be the higher decomposition rate of the formed Δ^9-THC in the oxidative ambient. An increased rate of Δ^9-THC was published in a cannabis resin sample exposed to air compared to that stored in a sealed plastic bag at ambient temperature, indicating the role of oxygen in Δ^9-THC decomposition.

Among THC isomers, Δ^8-THC has also psychotropic effects. The Δ^8-THC molecule formed by an additional isomerization of cyclization during thermal treatment. Both Δ^9-THC and Δ^8-THC were formed. By increasing the temperature, the relative yield of THC decreased, while other decomposition reactions became more pronounced.

In the present study, a notable amount of cannabinol was detected as a thermal degradation product of CBD. The most intense formation of cannabinol (10.4%) was observed at 400 °C in an oxidative atmosphere.

Cannabichromene also formed through cyclization reaction. Cannabichromene formation was more pronounced in an inert atmosphere compared to the oxidative condition at 250 °C. At higher temperatures, there were no significant differences, and the relative yield of cannabichromene was decreased.

Cannabielsoin was only detected under oxidative conditions. The relative intensity of cannabielsoin was around 3% and its quantity was not much affected in the temperature range of 250–500 °C. At higher temperatures, the share of the decomposition products formed through the cyclization reaction decreased, while the relative intensity of smaller molecules formed by C–C bond scission increased in the pyrolyzate.

4.3. Quantification of CBD content in food-supplements and hemp seed oils by UHPLC-UV methods

4.3.1. Label analysis of food products and food-supplements

Twenty-seven products were included in the analysis. Eighteen products were oils enriched with CBD, one product was a gelatine capsule, one product was an e-cigarette liquid, and five products were hemp seed oils. Because of the poor description of the ingredients the chemical characterization of CBD or hemp extract could not be identified from labels. Sixteen products

contained some kind of hemp extract, namely hemp flower extract, phytocannabinoid extract, various supercritical extracts, hemp seed extract, alcoholic extract, hemp extracts, CBD hemp extract, and full spectrum plant extract, cannabidiol hemp extract. Oils were added to the products. Hemp seed oil was the only declared ingredient in five products. The claimed CBD content was in the range of 10–50 mg in mL of oil or in one piece of capsule, and no CBD content was highlighted on the label of six products.

4.3.2. Validation

The goal of our work was to set up a properly validated method in order to reliably measure the cannabinoid content of the investigated food-supplements. The investigation included the analysis of CBD-enriched food-supplements from various sources along with preferable sample preparation.

Calibration curve and linearity

One of the major CBD cannabinoid markers was chosen to be analyzed and quantified. The calibration curve was adjusted to the 6 calibration points. The regression equation was y = 17205893410.02x – 335045.50 with correlation coefficient 0.9997. The calibration curve covered the range of 0.03–1 µg/injection. The values of LOD (0.02935 µg/injection) and LOQ (0.08895 µg/injection) were determined. The calibration curve covered two orders of magnitude of analyte concentration.

During the validation process the filter compatibility was determined using PTFE filter of 0.45 µm and 1.74% decrease of the investigated analyte was observed. The analyte seemed to be stability based on *stability* test: the sample stored at –20 °C and injected on days 0, 1, 5, and 7 resulted in on the first day 98.24%, on the fifth day 102.93, and on the seventh day 99.74% of CBD analyte compared to 0-day measurement. Based on these results, the maximum analyte reduction was 1.76%, thus the storage time did not affect the CBD content. The *system suitability* proved this method to be suitable for measurement (low RSD values of the area under the curve (AUC RSD%=0.27%), retention time (R_t RSD% = 0.17%) and the tailing factor range (1.066–1.089) calculated from five injections). Accuracy was evaluated based on recovery of CBD analyte using product sample CB24. The recovery values for the concentration levels of 50, 100, and 150% were 95.2–99.0% (RSD%=3.24%), 99.78–100.42% (RSD%=1.13%), and 96.64–99.32% (RSD%=0.48%), respectively. Injection of CB24 for ten times afforded the precision assessment of the system: the RSD% of AUCs was 3.89%. The repeatability based on CB24 analysis for six times, the RSD% for CBD value was 1.73%. The intermediate precision was determined by performing sample preparation by two chemists and evaluating the RSD% of obtained results, which was 4.01%.

4.3.3. Quantitative analysis of the CBD content

CBD was detected in products according to retention time and UV absorption (**Figure 5**). The quantification of CBD was performed using an external calibration curve. The CBD content of the products was presented graphically in **Figure 6a**.

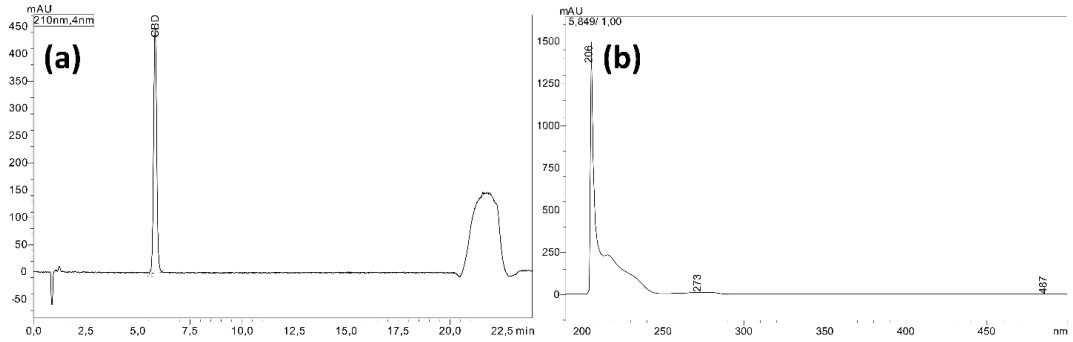

Figure 5. UHPLC chromatogram recorded at 210 nm (a) and UV absorption (b) of CBD.

In five products CBD could not be detected. Although in e-cigarette liquid (CB6) and in gelatine capsule (CB15) the CBD was present; however, the content of analyte was below the limit of quantification. In products CB1–CB5, CB8, CB9, CB16–CB27 the CBD content ranged from 12.87 to 54.09 mg/mL. Based on label analysis, the label accuracy was evaluated and classified as under-, accurately-, and over-labeled with detected CBD concentrations <90%, 90%–110%, and >110% of the labeled value, respectively. Information about the CBD content could not be found on the label of products -, therefore, the accuracy of the labeling could not be assessed. Two products were under-labeled, nine products were over-labeled, while eight products were accurately labeled (**Figure 6a**).

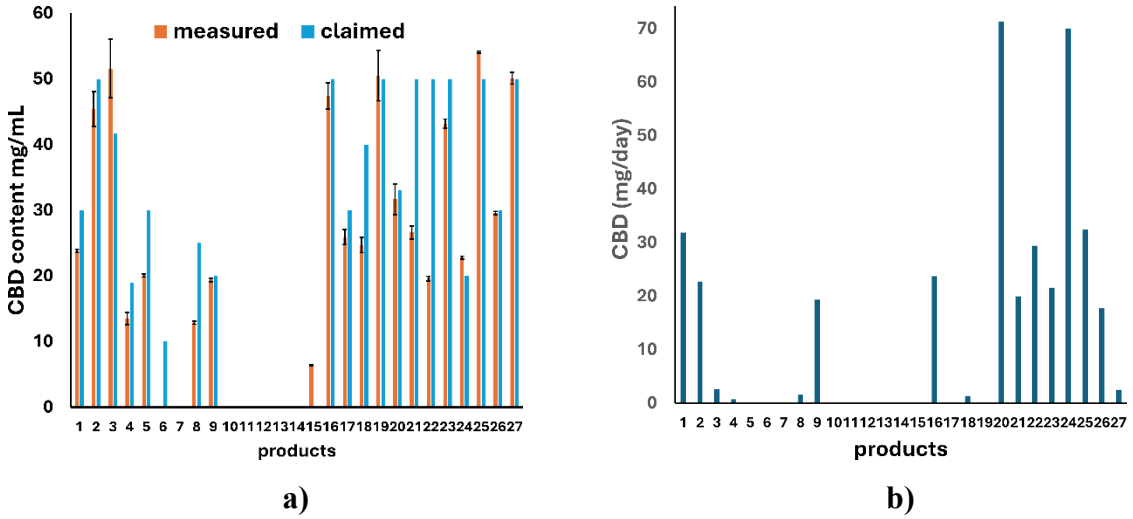

Figure 6. Declared and quantified CBD content of the product (a) and the daily CBD intake recommended by the manufacturers of the products (b). Values are presented as mean ± SD (n=3).

The daily intake of CBD was calculated according to the recommended daily consumption of the product (considering 1 drop equal to 0.05 mL) and the measured CBD concentration. The daily CBD intake was 1.24–71.26 mg (**Figure 6b**).

5. DISCUSSION

Cannabis sativa L. has long been known to people of all kinds. The application of the plant or its extracts vary in terms of safety and tolerability. The pure compounds of *Cannabis* have different effects depending on the molecular mechanism and individual sensitivity.

In the 1960s, cannabis was widely used as a cigarette, which made it relevant to study the pyrolysis of cannabinoids. Due to the complexity of the plant, performing experiments with the pure compound seemed like a suitable way to test the hypothesis. Nowadays, the investigation of the pyrolysis of cannabinoids is relevant because CBD is widely used in e-cigarettes, and previous studies have not analyzed the rate at which this compound is converted to other cannabinoids, such as the psychotropic THC. In our experiments, the mimicked environment was comparable with the one used in electronic cigarettes but did not exactly model the accurate chemical reaction mechanism. The most significant result was the demonstration that CBD is converted to THC in significant proportions by heat treatment, which sheds a special light on the use of CBD in e-cigarettes. THC is a known illegal substance that can lead to abuse and addiction. It has many well documented adverse effects ranging from dysphoria, hallucinations, and paranoia to milder form such as confusion, headache, euphoria etc.. It especially needs to be addressed for people who are unaware of the possible consumption of THC while they are driving or working with heavy vehicles. On top of that, the amount of CBD that is not transformed during pyrolysis creates another type of threat. In their article, Orvos et al. investigated the electrophysiological aspects of CBD both *in vitro* and *in vivo*. In the potential cardiovascular risks connected to CBD, hERG, and I_{Kr} channels play an important role. The application of CBD containing e-cigarettes without control may lead to an increased blood plasma level of CBD, thus it can trigger possible proarrhythmic adverse events. The occurrence of possible adverse events, based on the scientific results, may be especially high in people with impaired CBD metabolism and/or if the repolarization reserve is weakened.

In addition to its use as a recreational drug, *Cannabis sativa* has become increasingly important as a medicinal plant in recent decades, and its compounds and their derivatives are also marketed as medicines. The experience gained from the illegal use of the plant and the wide range of pharmacological effects of cannabinoids justify the question of whether the safety

of cannabinoid-based medicines can be considered acceptable. The efficacy of nabilone and dronabinol has been confirmed in several clinical trials and meta-analyses. However, data on safety and AEs are also necessary for the assessment of risk-benefit ratios. We presented the results of the first systematic review and meta-analysis on the AE profiles of nabilone and dronabinol based on the results of randomized, double blind, placebo-controlled trials. In case of nabilone, four AEs were meta-analyzed. Drowsiness, dizziness, and dry mouth were more frequent in the patients treated with nabilone than in the placebo group, whereas the frequency of headache was not different in the two groups. In patients treated with dronabinol, more adverse effects could be meta-analyzed. The frequency of dizziness, dry mouth and headache was significantly higher in the dronabinol groups, whereas in case of nausea, drowsiness, and fatigue no significant difference could be observed. Dizziness and dry mouth are common in case of the application of both pharmacons. Although the adverse effect profiles of cannabinoids have not been clinically compared with the therapies recommended by guidelines, based on the available evidence, the risk-benefit ratio of cannabinoids does not seem to be inferior. Further, high-quality trials of appropriate patient size, examining the adverse-effects of dronabinol or nabilone with comparable and more uniform endpoints would allow to assess the safety profile of these compounds with a lower risk of bias. Moreover, a considerable number of trials reporting the same or similar adverse-effects that can be easily grouped and that are related to different doses of these drugs would enable the assessment of the dose dependency of the adverse-effects.

Cannabinoids are used not only as medicines but also as food-supplements. The most popular is CBD, which is promoted for its many medicinal properties, however, only a few applications are evidence-based. The vast majority of products with CBD content designed for oral administration belong to unregulated products. Due to a lack of information on the long-term effects of CBD consumption and reliable data on CBD toxicity, the authorities chose a restrictive position by not recommending these products for consumption. Uncertainties regarding toxicity and CBD-related adverse effects are further supported by controversial data reported by recent clinical trials, however, other clinical studies with the same dose have not observed these unwanted events. The reported data support worries of the FDA warning letters claiming that a safe dose of CBD has not been established yet.

Another possible risk source for CBD-containing food-supplements or foodstuffs could be the uncertainty about the composition of products. Generally, there are three major types of CBD-preparations: full-spectrum CBD, broad-spectrum CBD, and CBD isolate products. The risk of non-psychoactive phytocannabinoids in these products, besides CBD, is also unknown,

since there are no data on their toxicity or safety in long-term consumption. A study that examined THC contamination of unregulated CBD-containing products revealed that consumption of THC-free labeled products might lead to unintentional exposure to THC and related side effects.

The CBD content of various products has been analyzed by several research groups to determine the accuracy of the labeling. An analysis carried out in South Africa revealed that only 7.5% of the investigated products (n=3/40) have a CBD content in the range of 90% to 110% of their claim on the label, while two oils were under-labeled with +27.48% and +49.42% CBD content, respectively. In the United States, 84 products have been purchased online. Quantification of the CBD content of these products revealed that 30.95% of the analyzed products were accurately labeled (n=26/84) and 42.85% contained more CBD than claimed on the label (n=36/84). There are identified problems in the regulation of dietary supplements that go beyond the shortcomings in the regulation of e-cigarettes, and our research has confirmed this. The loose control over food-supplements in the EU and in Hungary brings along the appearance of poor quality or not adequately inspected products Performing a quantitative analysis of a bunch of CBD-containing products in Hungary suggests that the quality of the products vary. Labeling accuracy tends to have a large deviation. The analysis of the purchased products shows a surprising picture that relates the prices of these products and the illustrated beneficial claims on the websites of the distributors. The analytical method developed combines the features found on various websites of column manufacturers and the data found in the analytical literature of the field. Therefore, it has the potential for easy applicability, robustness, and reliability. UV detection limits the quantification of minor compounds in the analyzed products. From this point of view, for the analysis of minor compounds, a coupled instrument might count as a better choice.

Our applied UHLPC-UV analytical method was suitable for our investigations of CBD-containing food-supplements and hemp oils. Further analytical research and a better understanding of consumer attitudes can lead to safer applications and improved analytical methods towards quality control. Our findings suggest that analyzed hemp seed oils are free of phytocannabinoids. The analyzed food-supplements were mostly not accurately labeled and without a clear description of the origin of CBD and possible phytocannabinoid content. Our study provides further evidence that the safety issues of CBD-containing food-supplements must be addressed by authorities, and restrictive regulations are justified.

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