Evaluation of medicinal plant-based products used for weight loss

Summary of Ph.D. Thesis

Dorottya Koncz

Institute of Pharmacognosy

University of Szeged

Szeged

2023

University of Szeged
Doctoral School of Pharmaceutical Sciences
Head: Prof. Judit Hohmann DSc
Pharmacognosy Doctoral Programme
Programme director: Prof. Judit Hohmann DSc.
Institute of Pharmacognosy
Supervisors:
Prof. Dezső Csupor DSc
Dr. Barbara Tóth PhD

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Complex Exam Committee

Head:	**Prof. Judit Hohmann DSc**, Institute of Pharmacognosy, University of Szeged
Members:	**Prof. Ildikó Csóka PhD**, Institute of Pharmaceutical Technology and Regulatory Affairs, University of Szeged
	Dr. Gábor Janicsák PhD, Hungaropharma PLC

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Head:	**Prof. Ildikó Csóka PhD,** Institute of Pharmaceutical Technology and Regulatory Affairs, University of Szeged
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Szeged
2023

1 INTRODUCTION

Since obesity has become a global epidemic leading to increased morbidity and mortality, finding safe and effective ways to lose weight and prevent obesity are of utmost importance. To reverse unintended changes in body composition, lifestyle and dietary adjustments are generally advised; nevertheless, it is well known that in this case it is much easier said than done. Therefore, the majority of those who struggle with obesity yearn for quick and secure solutions; yet, there are few, if any, medicines that can address this problem in the long run without having considerable side effects. Several medicines have been withdrawn due to safety issues, and only a few active pharmaceutical ingredients (APIs) are marketed for weight loss and these can only be used in only in severe cases, like obesity and coexisting metabolic disorders.

Since medicines are not easily available in this indication, patients prone to try alternative solutions. Therefore, it is not surprising that obese patients turn to readily products regardless to their questionable quality, and the popularity of food supplements for weight loss management is increasing. This phenomenon gives a rise to several problems including patient safety. It has been proven that food supplements marketed for weight loss frequently contain illicit sympathomimetic drugs that are concealed to increase efficacy. Another common way to increase the efficacy is to use an ingredient above its allowed upper limit (UL). Therefore, the actual composition of several weight loss products tends to differ from that on the label. Due to the flaws in the regulation of food supplements, these products may also be contaminated (e.g. with heavy metal, microorganisms, and pesticides). Moreover, the lack of a clinically validated safety and efficacy profile raises doubts about the efficacy of a number of herbal ingredients. The RASFF (Rapid Alert System for Food and Feed), a crucial tool for providing important information about unlawful goods, issues the notifications regarding counterfeit food supplements. Reviewing the RASFF system allows to map current counterfeit tendencies and to collect the main adulterants occurring in weight loss supplements. Data extracted from the RASFF enables to categorize the most common quality issues.

2 AIMS OF THE STUDY

The aim of this work was to

- systematically review the European Rapid Alert System for Food and Feed (RASFF) reports to map the tendencies in food supplements / illegal products that are used to promote weight loss,
- based on the warnings collected via the RASFF, map the unauthorized substances dominating the market,
- to mark the mainstream signals as a public health issue, to initiate possible solutions to reduce counterfeit,
- to summarize the available and previously withdrawn active pharmaceutical ingredients in the European Union,
- to collect the most popular herbal food supplements and summarize their safety and efficacy,
- to compare the attributes of synephrine and ephedrine (a previously banned food supplement ingredient) in a systematic review, and
- to conduct a meta-analysis on synephrine to determine its proper utilization based on scientifically established results.

3 MATERIAL AND METHODS

3.1 Analysis of RASFF data

Data from the RASFF portal were collected retrospectively. The filter in the portal was set for "dietetic foods, food supplements, fortified foods". Data from individual reports were entered [date, product, product category, notification type, origin (notified by), countries concerned, subject, action taken, distribution status and risk decision]. Notification records were sorted into four main categories. The first was "A" for unauthorized ingredients; the second represented "B" for unsafe ingredients; and two others: "C" if there was a problem with the level of the ingredient (too high or too low) or "D" which meant other problems (e.g. mislabelling, taste disturbance). "A" category was handled differently than "C", and the results were analysed also separately. As part of the RASFF Portal, RASFF signals are categorized as alert, information notification, or border rejection. On the basis of the reported product's intended application, subcategories were constructed. The risks and adverse effects were also evaluated. Before January 1, 2020, all data from the reported supplements database, ranging from 1988 to 2019, were extracted. Each entry was individually reviewed. Descriptive analyses were performed using Microsoft Excel 2010 (Microsoft Excel, RRID:SCR_016137) for Windows (Microsoft Inc.), after categorizing RASFF signals.

3.2 Meta-analysis

Literature search and selection criteria

The Cochrane Library, PubMed, EMBASE, and Web of Science (WoS) databases were searched electronically. All databases were checked until August 17, 2022. The key term 'synephrine' was used in the search process. Following the CONSORT recommendations, this meta-analysis of eligible peer-reviewed articles was presented in compliance with the PRISMA statement. Trials were chosen if they met the following criteria: (1) they involved humans, (2) they compared known doses of orally administered synephrine with a placebo, an active control, or both, and (3) they were completed. The PICO (patients, intervention, comparison, outcome) format was used to accomplish this work as follows: P: Adults; I: known *p*-synephrine dosage administered orally; C: placebo or control; and O: changes in body weight, composition, cardiovascular, and metabolic markers [i.e., heart rate (HR), blood pressure, body weight, body fat, fat mass, fat-free mass, fasting blood sugar level, and respiratory exchange ratio (RER)

values]. Our hypothesis was that synephrine promotes weight loss and that using it is linked to undesirable cardiovascular effects. This work is PROSPERO registered (359626). There were no restrictions on the number of patients who could participate or the amount of *p*-synephrine that could be administered. The included articles had to be accessed in English.

Data extraction and endpoints

Only clinical trials involving adults were included in this meta-analysis, as stated in the PICO. Findings related to the efficacy and safety of synephrine were collected from these clinical trials. The values that were available at least in three articles were chosen for the final analysis of the study endpoints. The final trials/outcomes of analysis could change as a result of statistical analysis. The primary author's name, publication year, study design, population, participant count, other medications used in the intervention group, synephrine regimen, and results were all taken out of individual studies.

Quality evaluations

The literature search was performed independently by two authors (Dorottya Koncz and Dezső Csupor), and both authors read the full-text articles and extracted the relevant information out of the sources. The Cochrane Risk of Bias Tool, which includes the following domains: random sequence generation, allocation concealment, the blinding of participants and personnel, the blinding of outcome assessment, incomplete outcome data, selective reporting, and other scores of bias, was used performed by two of the authors (Dezső Csupor and Barbara Tóth.) to analyse the risk of bias. Studies were classified as having a high (red), unclear (yellow), or low (green) risk of bias for each domain. Any disagreements finally were resolved by consensus. The Review Manager (RevMan) software from the Cochrane Training site in London, UK was used for generating risk of bias figures.

Statistical analysis

If an outcome was mentioned in at least three papers, it was chosen for the final analysis; nevertheless, subsequent attrition and particular time periods could change the examined study and outcome number. A post-post analysis was conducted to evaluate the weighted mean difference (MD) and effect sizes (ESs) between test and control group values (synephrine vs control). The heterogeneity was assessed using Chi^2 or Tau^2 tests. When there was any additional intervention(s) beyond synephrine, the varying study intervention arms were

eliminated from the final analysis (mainly caffeine). In the case of subjects who consumed different amounts of caffeine, habitual low caffeine users were chosen compared to regular high caffeine users. The Review Manager 5.4.1 program was used to carry out the statistical analysis. When the p value was less than 0.05, the findings were accepted statistically significant.

4 RESULTS AND DISCUSSION

4.1 Analysis of RASFF notification data

2,559 records of food supplements with quality issues were included in the RASFF database's raw data set (**Figure 1**). Our research focused on products with an intended use as a slimming agent [372 (14.54 %)]. 319 (12.5%) of the overall counterfeited weight loss products were containing illegal unauthorized substances ("A" category). Firstly, we summarized the trends of illegal food supplements related with warning signals in RASFF between 1988 and 2019. The products reported for suspected quality issues (e.g. adulteration) are potential sources of serious risks. Hence, these products may cause serious adverse effects and dangerous interactions in sensitive patients. We collected and summarized the most frequently used unauthorized natural and synthetic adulterants and assessed the trends of adulterated and illegal food supplements based on the warnings of the RASFF. This work was designed to help risk management and risk minimization concerning food supplements. The rising frequency of illegal food supplement signals in RASFF is alarming**.**

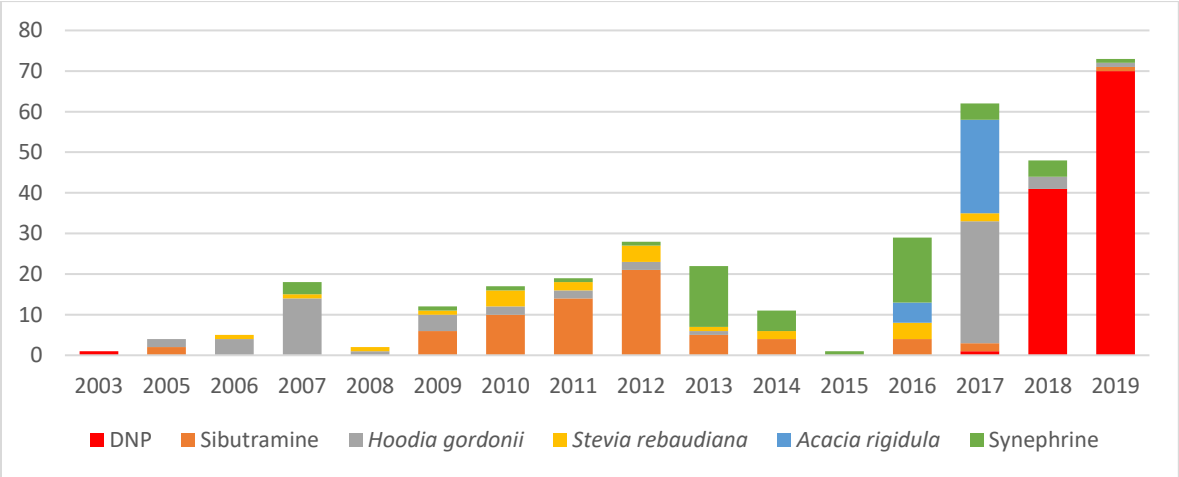


Figure 1. Notifications on weight loss food supplements in the RASFF (2003–2019) (Accessed 2019.12.31.)

Between 1988 and 2019, 2,4-dinitrophenol [DNP, 113 out of 319 (35.4%)] and sibutramine (69 products, 21.6%) were the most frequent illegal substances in weight loss products. The former may cause rapid weight loss, but serious side effects are related to its use. In the past, numerous deaths linked to DNP have been reported. DNP was identified as an adulterant for the first time in 2003, however between 2017 and 2019, more products containing this compound have been reported. Products with DNP-contamination were primarily found in the United Kingdom.

Although sibutramine was reported from several countries, there were fewer products that included it. Based on the Sibutramine Cardiovascular Outcomes Trial (SCOUT) trial, sibutramine increases the risk of nonfatal myocardial infarction and nonfatal stroke in individuals with pre-existing cardiovascular disease and increases the likelihood of high blood pressure or rapid heartbeat. It is alarming, that a large percentage of the reported signals were associated with hazardous synthetic drugs like DNP, sibutramine and phenolphthalein. DNP has been the subject of an increasing number of reports, and as in the medical literature, the lethal oral dose is about only 1-3 g *per os*, and 3 g has proven fatal, even in divided doses over a period of 5 days. Although the dosages from the fatal reports vary from 300 mg to 10 g, there is no safe level of DNP. Therefore, DNP should be monitored more closely in the future. As the notifications accessed in the RASFF system did not contain the dosage of DNP in the offered food supplements, it must be taken even more seriously as the majority (99%) of the reports were in serious risk category.

Among the most popular food supplements of natural origin, extracts of *Hoodia gordonii* [66 of 117 (56.4%)], *Stevia rebaudiana* (23, 19.66%) and *Vachellia rigidula* (syn.: *Acacia rigidula*) (28, 23.93%) were the most frequently registered illegal items in RASFF from 1988 to 2019. Based on the results of our research of RASFF, *Stevia rebaudiana* appears to be the least harmful and least risky ingredient and it was reported in a modest amount. Even though it has been used as a traditional medicine for hundreds of years, additional scientific and clinical research is still required to confirm its safety because it was almost consistently represented in RASFF from 1988 to 2017 and is extremely popular by customers. The safety of the other two plants (*H. gordonii* and *A. rigidula*) has not been established scientifically, and they are still not accepted as novel foods. Despite the fact that *H. gordonii* is frequently used as an adulterant and promoted for its ability to aid weight loss, little is understood about its chemical components and their action mechanism. Recent studies revealed that taking *H. gordonii* may raise blood pressure and heart rate. *H. gordonii* has been resurfacing often since 1988, it is obligatory to monitor food supplements that include *Hoodia*. Several biogenic amines can be found in the native shrub *A. rigidula*, which grows in the South-eastern United States. The plant has been utilized for weight loss products, but neither its traditional use – it has never been applied in traditional medicine – nor the literature data have yet provided evidence of its effects. Recent occurrence of *Acacia rigidula* in the RASFF portal emphasizes the need to monitor *A. rigidula* in dietary supplements. Because there is no legal regulation that limits the amount of synephrine and other alkaloids in dietary supplements, each country should set a maximum quantity of synephrine. Because synephrine has a daily dose limit in some countries, there have

been constant reports in the RASFF about products containing more synephrine than allowed. Overall, 53 out of 372 reports (14.25%) were recorded in connection with synephrine in "C" category (16 in 2016 and 15 in 2013).

4.2 Efficacy and safety of synephrine and *C. aurantium* extracts

Although its efficacy and safety have not been fully demonstrated, *p*-synephrine, a protoalkaloid isolated from the immature fruit or peel of the bitter orange (*Citrus* x *aurantium*), is extensively utilized in weight reduction and sports performance products. We aimed to determine the efficacy and safety of synephrine in order to dispel myths surrounding its use because it appears that legal background and notification procedures do not always guarantee safety and frequently lack scientific evidence to ensure efficacy. Synephrine is frequently mentioned in RASFF in connection with UL issues.

To evaluate synephrine's effects on weight loss and determine its safety based on its effects on cardiovascular variables, different time sets had to be applied depending on the original time points in each outcome. According to the literature, the range of daily doses for *p*-synephrine is 25 to 100 mg, but participants in the included trials took 6–214 mg daily. This meta-analysis examined 341 adult participants from 18 trials in total. Our systematic review and meta-analysis revealed that *p*-synephrine had no effects on body weight or composition measures, but it did raise blood pressure over time.

Systolic blood pressure

According to our meta-analysis, synephrine significantly elevated systolic blood pressure when taking for prolonged time, but it did not have such effects acutely (p=0.02) **(Figure 2).** To evaluate the effects of synephrine on blood pressure, a total of 11 trials with 222 participants, 6 different time sets, and various dosages (range 10–214 mg) were used. The synephrine group's systolic blood pressure (SBP) tended to increase. The mean difference was 1.21 mmHg (95% CI: -2.57–5.00), which was not significant (p=0.53) but demonstrated a larger effect size for synephrine than for placebo 30–45 minutes after the administration of 20–50 mg synephrine. The post-post analysis revealed a non-significant increase in SBP (MD 1.56 mmHg, 95% CI: -1.11–4.24, p=0.25) following the administration of the products containing 49–214 mg synephrine for with an hour. Based on the results of to five studies including 61 patients, the effects of synephrine (49–180 mg) remained insignificant after two hours, (MD 3.89 mmHg, 95% CI: -0.99–8.77, p=0.12). Only a small effect on SBP was seen after 3 hours after

administration (MD 0.34 mmHg, 95% CI: -2.18–2.87, p=0.79), and it diminished after 6–8 hours from consumption (MD 0.10 mmHg, 95% CI: -3.82–4.02, p=0.96; dosage 27–108 mg). Based on the results of two trials involving 75 patients, after 8 weeks of administration, a daily dose of 10–49 mg synephrine had a significant effect on the systolic blood pressure (MD 6.37 mmHg, 95% CI: 1.02–11.72, p=0.02).

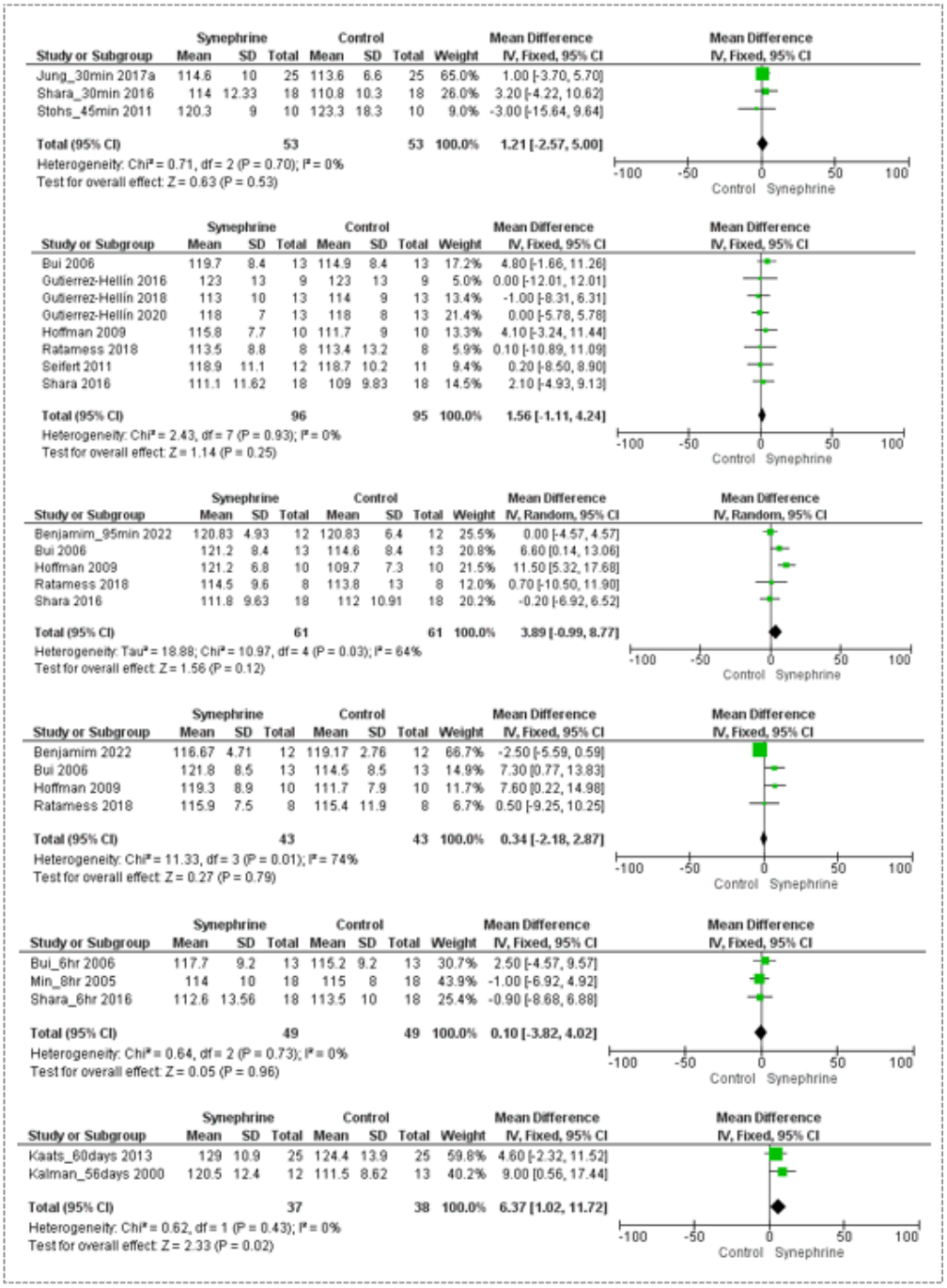


Figure 2. Forest plot diagram of synephrine on systolic blood pressure acutely (after 30–45 min; 1 h; 2 h; 3 h; and 6–8 h) and long duration (56–60 days) in the intervention and the control groups

Diastolic blood pressure

When used for extended periods of time (8 weeks), it demonstrated similar significant effects and less pronounced acute effects on diastolic blood pressure (DBP, p=0.03) (**Figure 3**). Synephrine (20–214 mg) administration did not significantly affect DBP neither 30–45 minutes after administration (MD -0.88 mmHg, 95%CI: -4.45–2.70, p=0.63), nor 1 hour after administration (MD -0.89 mmHg, 95%CI: -2.92–1.13, p=0.39), nor 2 hours after administration (MD 0.48 mmHg, 95%CI: -2.22–3.17, p=0.73); nor 3 hours after administration (MD 0.40 mmHg 95%CI: -1.83–2.62, p=0.73); nor 6–8 hours after administration (MD -0.43 mmHg 95%CI: -3.52–2.66, p=0.78). A significant effect on DBP was observed after long-term (8 weeks) use of synephrine (10–49 mg) (MD 4.33 mmHg, 95% CI: 0.48–8.18, p=0.03), based on two trials with 75 participants.

Heart rate

The participants' HR increased following acute synephrine administration, although the difference remained insignificant; the biggest elevation was detected 3 hours after ingestion (p=0.07). Overall, the effects of synephrine on HR were investigated in 9 studies with 129 participants, 6 different time periods, and varying synephrine dosages (range 20–214 mg). Significant differences between synephrine and placebo were not detected; nevertheless, the HR in the synephrine group modestly rose 30 minutes to 6 hours after consumption. The mean difference in HR was 3.15 beats per minute (30–45 minutes) after taking 20–50 mg synephrine (95% CI: -0.41–6.71, p=0.08), and it was 1.11 beats per minute (95% CI: -1.32–3.53, p=0.37) after 1 hour of taking 49–214 mg synephrine. A non-significant increase in HR was seen 2 hours after ingesting 49–180 mg of synephrine (MD 3.15 beat/min, 95% CI: -0.65–6.96, p=0.10). Based on four studies involving 43 subjects, the synephrine-adjusted increase in HR was 3.48 beat/min (95% CI: -0.33–7.29, p=0.07) 3 hours after ingesting 60–180 mg of synephrine. Based on the included studies, the effects were still not significant after 4 hours (MD 3.25 beat/min, 95% CI: -2.86–9.35, p=0.30), or after 6 hours (MD 2.84 beat/min, 95% CI: -2.80–8.48, p=0.32; range 49–108 mg).

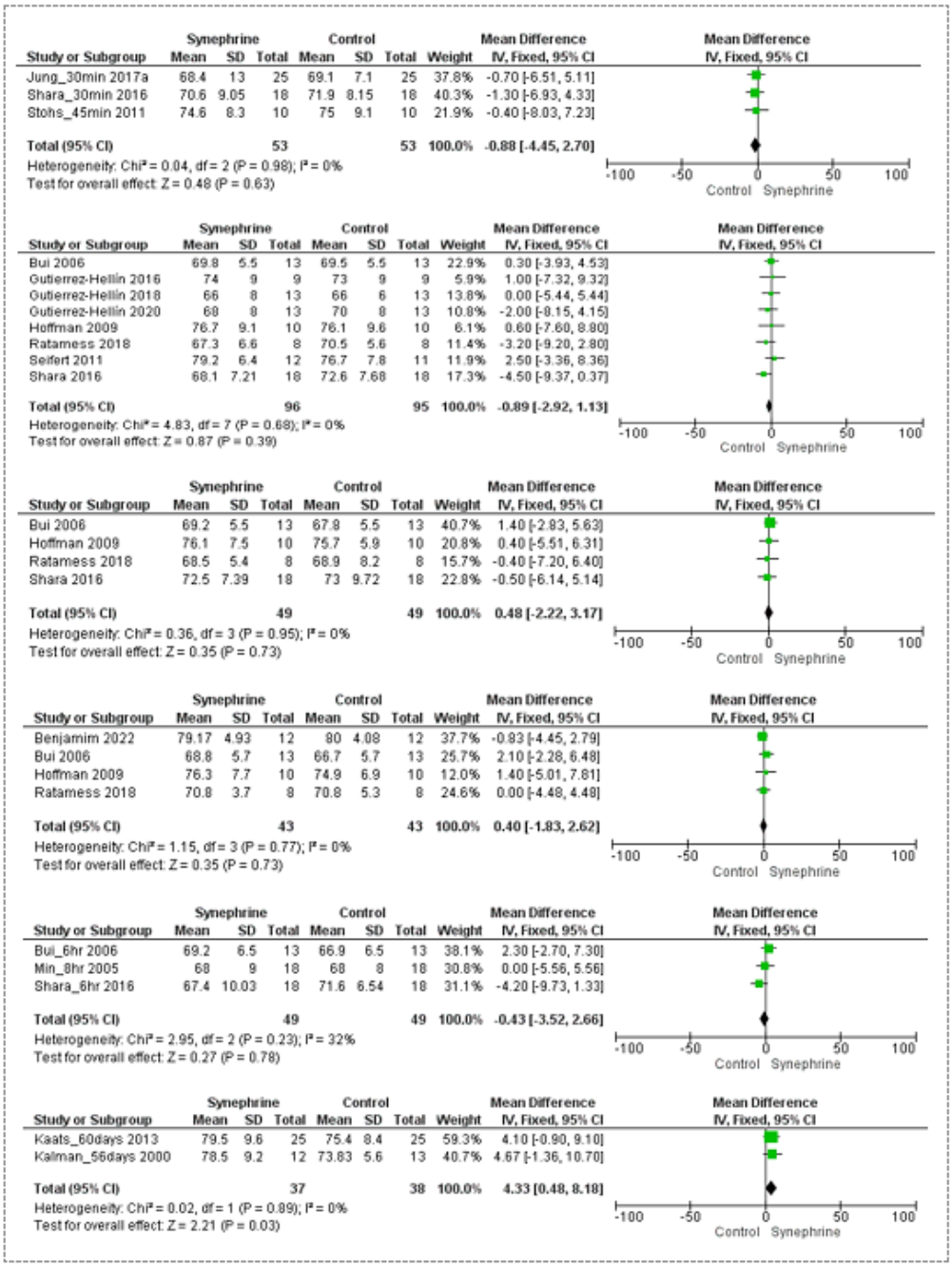
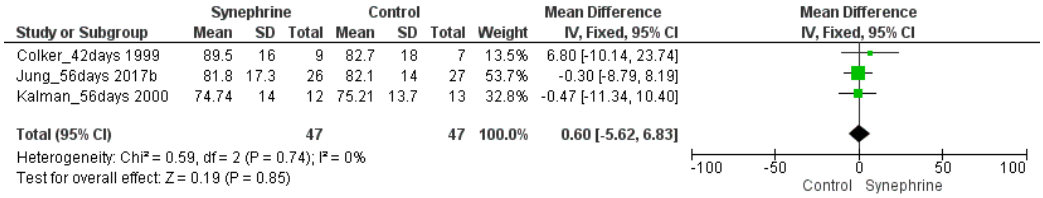


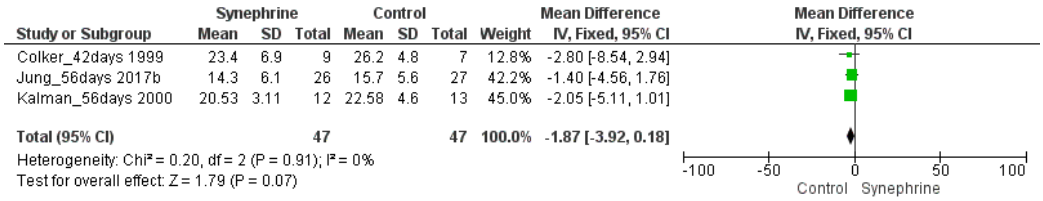
Figure 3. Forest plot diagram of synephrine on diastolic blood pressure acutely (after 30–45 min; 1 h; 2 h; 3 h; and 6–8 h) and long duration (56–60 days) in the intervention and the control groups

Weight loss and body composition

Our investigation led to the conclusion that prolonged use of 10–54 mg synephrine (6–8 weeks) resulted a not significant change in body weight (MD 0.60 kg, 95% CI: -5.62–6.83, p=0.85) (**Figure 4A**), body fat (MD -1.87%, 95% CI: -3.92–0.18, p = 0.07) (**Figure 4B**), fat mass (MD -0.32 kg, 95% CI: -3.76–3.11, p=0.85) (**Figure 4C**), and fat free mass (MD 0.47 kg, 95% CI: -4.19–5.13, p=0.84) (**Figure 4D**), which were all similarly unaffected by synephrine in the analysed 3 trials.



(A)



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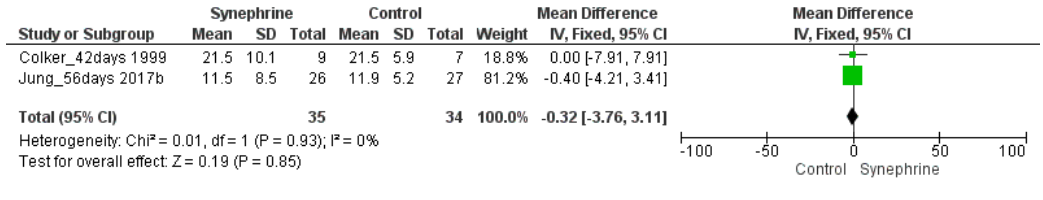
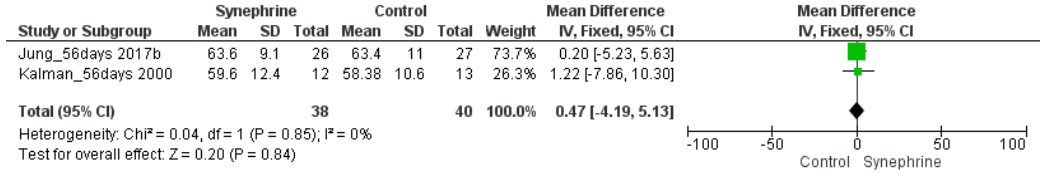


(C)



(D)

Figure 4. Forest plot diagram of synephrine on weight (A), body fat (B), fat mass (C), and fat free mass (D) after 42–56 days (6–8 weeks) in the intervention and in the control groups

Other outcomes

Based on the included three studies, 6–103 mg synephrine did not significantly affect blood glucose levels. After 2–3 hours of the consumption of synephrine, blood glucose levels increased slightly but not significantly (MD 4.62 mg/dL, 95% CI: -3.04–12.29, p=0.24) with moderate heterogeneity (TAU^2= 30.32%).

Three studies were conducted to examine the acute effects of synephrine (6–60 mg) on RER. After 1, 2, or 3 hours of dosing, synephrine had no significant effects. After one hour of consumption, there was no difference [the mean difference was 0.00 (95% CI: -0.03–0.03, p = 0.91)]; strengthened by a non-classical leave one out analysis; after two hours, synephrine produced a difference of -0.02 (95% CI: -0.12–0.09, p=0.75; after three hours, a difference of -0.02 (95% CI: -0.12–0.08, p=0.73) was observed.

The use of ephedrine is linked to an elevated risk of cardiovascular morbidity and mortality; therefore, a comparably potent but more secure substitute would be greatly appreciated. However, our results clearly indicated that the use of synephrine may also have negative impact on the overall cardiovascular health. The daily dosage of synephrine employed in the analysis of weight loss was 10–54 mg for 42–56 days, which is almost the same as the dosage that caused a cardiovascular adverse event (increased blood pressure) 56–60 days following the administration of 10–49 mg synephrine. Our meta-analysis showed that using synephrine also hinders the cardiovascular health; therefore, it might not be a safe substitute for ephedrine for people who have pre-existing comorbidities. Moreover, there was no evidence on its weight loss and other beneficial properties.

5 CONCLUSIONS

Given the ongoing global expansion of the food supplement sector, it is critical to understand the potential for counterfeit products to pose a public health risk. Counterfeit signals are important tools to make food supplements safer for the consumers, and the protect their health. It is necessary to establish a number of initiatives to reduce the prevalence of counterfeit products by strengthening industry standards for testing efficacy and safety. On the other hand, there is a clear need for efficient solutions to help weight loss because currently there are only a few accessible treatment choices.

Unpredictable interactions between easily available counterfeited food supplements and other medications can also lead to significant adverse events. It is concerning that it seems that there are increasing numbers of signals in RASFF about contaminated products. The RASFF contained overall 372 records for weight loss food supplements with a quality concern between 1988 and 2019. 319 (85.75%) contained unauthorized ingredients such as DNP [113 out of 319 (35.4%)] and sibutramine (69 (21.6%). There were less reports of unauthorized herbal substances [117 out of 319 (36.68%)]. In the category of inappropriate dosages, synephrine was identified 53 [53 out of 372 (14.25%)] times, as the synephrine content in these products was often over the authorized level in some countries.

Based on our observations synephrine cannot be considered as a safe and effective alternative for *Ephedra sinica* Stapf or ephedrine in the dose range of 10–214 mg. Furthermore, our meta-analysis identified safety issues with synephrine consumption. When used for longer periods of time, *p*-synephrine significantly raised both systolic and diastolic blood pressure and it also tended to elevate heart rate and systolic blood pressure acutely. Our meta-analysis revealed that it had no beneficial effects on body composition or weight loss. Consequently, the cost/benefit ratio of its use is unfavourable. The regulatory authorities, the industry and consumers must re-evaluate their perspectives on the balance between product quality and efficacy regarding food supplements as the current legal situation does not prevent all potentially dangerous weight loss substances from entering the market. My thesis only touches on a small portion of the vast and intricate problem of counterfeit food supplements.

ACKNOWLEDGEMENT

The person who I owe the greatest and sincere gratitude is my supervisor Prof. Dezső Csupor, who accepted and supported my scientific aims, ideas, and field while patiently and carefully guided me on the path through academic science. Without him and his trust, the entire project could not have been completed and probably would not exist.

I am very fortunate that I was able to learn from Dr. Barbara Tóth, who was my co-supervisor, during my PhD studies. I cannot express enough how much I appreciate all her support and expertise as my co-supervisor.

They were a constant source of inspiration and education for me. Both of them gave me narrow scientific knowledge that I will always remember and practice, enabling me to continue my scientific goals and keep this aspect in the future. Their encouragement, help and humanity made my scientific research succeed.

I am very grateful for Prof. Judit Hohmann who provided me the opportunity to be a part of the university and made my experiments and research possible.

I also thank my co-authors, Dr. Orsolya Roza, Dr. Tivadar Kiss and Dr. Muh. Akbar Bahar and to my colleagues in the Institute of Pharmacognosy: Dr. Ákos Bajtel, Balázs Kovács, Dr. Dóra Rédei, Attila Horváth, and Klára Horváth-Boros, their collaboration in our articles.

My parents have always encouraged and supported me in my scientific career, and I followed them when choosing biology and chemistry as my areas of interest. They played an enormous role in my PhD, which I want to underline and express my love and gratefulness in this paragraph.

My fiancé, who gives my life and work an essential purpose and is my partner in both science and life made my attitude and conception complete.

THE THESIS IS BASED ON THE FOLLOWING PUBLICATIONS:

I. **Koncz, D**., Tóth, B., Roza, O., & Csupor, D. (2021). A Systematic Review of the European Rapid Alert System for Food and Feed: Tendencies in Illegal Food Supplements for Weight Loss. *Frontiers in pharmacology, 11,* 2465. IF: **5.988**

II. **Koncz, D**., Tóth, B., Bahar, M. A., Roza, O., & Csupor, D. (2022). The Safety and Efficacy of *Citrus aurantium* (Bitter Orange) Extracts and p-Synephrine: A Systematic Review and Meta-Analysis. *Nutrients*, *14*(19), 4019. IF: **6.706**

The cumulative impact factor of the publications related to thesis: 12.694

OTHER PUBLICATIONS RELATED TO THE THESIS:

I. **Koncz, D**., Tóth, B., Kiss, T., Roza, O., & Csupor, D. (2021). *Acacia rigidula* versus other *Acacia* taxa: An alarming issue in the European novel food regulation and food supplement industry. *Acta Pharmaceutica Hungarica, 91*(2), 67-74.

II. **Koncz, D**., Tóth, B., & Csupor, D. (2021). Fogyasztószerként használt étrend-kiegészítők: Minőségi problémák az Európai Unióban. *Gyógyszerészet, 65*(11), 671-675.

III. **Koncz, D.** (2021). Reports of illegal food supplements for weight loss in the European Rapid Alert System for Food and Feed (RASFF). *In: Symposium of Young Researchers on Pharmacognosy* (Book of Abstract), 17.

IV. **Koncz, D**. (2022). Dangerous and Potentially Dangerous Components of Weight Loss Products. *In: Symposium of Young Researchers on Pharmacognosy* (Book of Abstract), 15.

V. **Koncz, D**., Tóth, B., Roza, O., & Csupor, D. (2020). In: *9th Interdisciplinary Doctoral Conference 2020* (Book of Abstracts), 108.