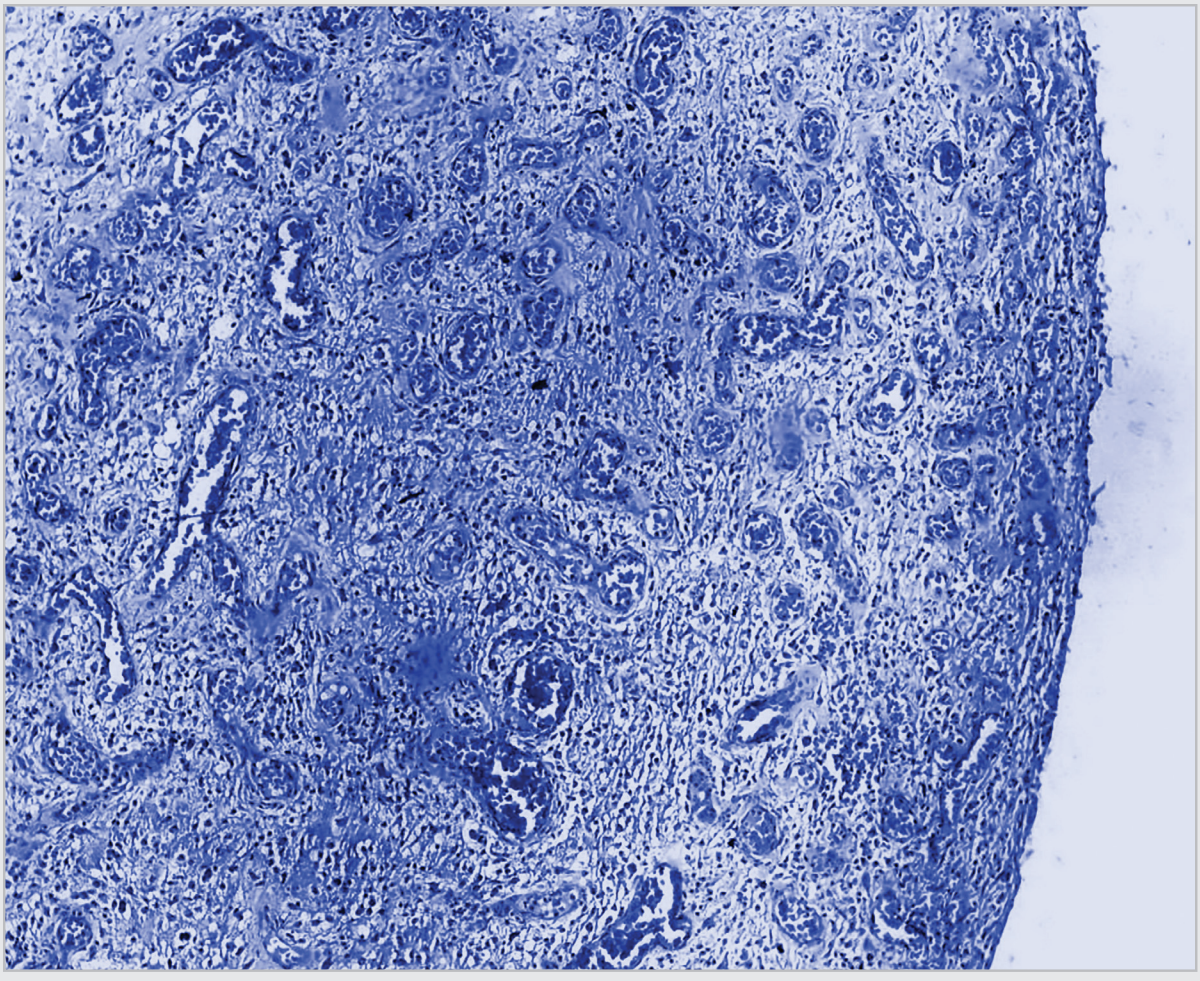
aph-hsps.hu

APH 2020;90:
1–32

1
2020

Acta Pharmaceutica Hungarica

Scientific Journal
of the Hungarian Society
for Pharmaceutical Sciences



Established in 1925. by Pál Lipták

APH 2020;90:1–32. **ISSN 0001-6659**

aph-hsps.hu

aph

Acta Pharmaceutica Hungarica

APH 2020;90
1-32.

Scientific Journal
of the Hungarian Society
for Pharmaceutical Sciences

1

2020

Contents

Márió Gajdács, Edit Paulik, Andrea Szabó: Public knowledge, attitude and practices towards antibiotics and antibiotic resistance: a cross-sectional study in Szeged District, Hungary – Public knowledge toward ABs … … … … … 5

Sarah Kehili , Mohamed Nadjib Boukhatem, Asma Belkadi, Faiza Boulaghmen, Mohamed Amine Ferhat, William N. Setzer: Spearmint (Mentha spicata L.) essential oil from tipaza (Algeria): in vivo anti-inflammatory and analgesic activities in experimental animal models … 15

Péter Gieszinger, Gábor Katona, Piroska Szabó-Révész, Rita Ambrus: Stability study of nasal powder formulation containing nanosized lamotrigine … 27

Acta Pharmaceutica Hungarica: www.aph-hsps.hu

„Acta Pharmaceutica Hungarica" Scientific papers of the Hungarian Society for Pharmaceutical Sciences
Published by the Hungarian Society for Pharmaceutical Sciences, represented by *Prof. Éva Szökő*
Gyulai Pál u. 16., Budapest, 1085 Hungary, Phone: (+36-1) 235-09-99;
E-mail: office@aph-hsps.hu
Subscibtion: Hungarian Society for Pharmaceutical Sciences, Gyulai Pál u. 16., Budapest, 1085 Hungary by international banktransfer
(OTP bank account number: 11708001-20530530) – Mailing address: 1447 Budapest, Pf. 480
Informations for international banktransfer:
Account holder: OTP Bank Ltd. – Nádor utcai Kereskedelmi Banki Centrum
Account Number: 11708001-20530530
Bankadress: H-1051 Budapest, Nádor u. 6.
IBAN: HU20 1170 8001 2053 0530 0000 0000
Swift Code (BIC): OTPVHUHB
Subscibtion fee: HUF 6000 + HUF 300 VAT
Published quaterly
Typesetting: *Csaba Oláh*
Printing: ColorToys Bt.

A jövő kötelez

CONGRESSUS PHARMACEUTICUS HUNGARICUS XVI.

1924 – 2020

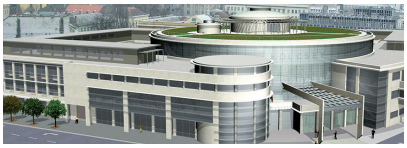
Debrecen, 2020. szeptember 10-12.

CONGRESSUS PHARMACEUTICUS HUNGARICUS

A jövő kötelez

DEBRECEN

2020. 09. 10–12.



A jövő gyógyszerészi kompetenciái • Innováció a gyógyszerészeti gyakorlatban Innováció a gyógyszerkutatásban és fejlesztésben • Információs technológia a gyógyszerészet területén

Részletes információk a kongresszus weboldalán elérhetők:

www.cph2020.hu

A CPH XVI. KONGRESSZUSÁT 2020. SZEPTEMBER 10-12. IDŐPONTRA HALASZTJUK VÁLTOZATLAN HELYSZÍNEN ÉS FELTÉTELEKKEL.

Public knowledge, attitude and practices towards antibiotics and antibiotic resistance: a cross-sectional study in Szeged District, Hungary

Public knowledge toward ABs

MÁRIÓ GAJDÁCS[1,2*], EDIT PAULIK[3], ANDREA SZABÓ[3]

[1]*Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, Szeged, Hungary*
[2]*Institute of Medical Microbiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary*
[3]*Department of Public Health, Faculty of Medicine, University of Szeged, Szeged, Hungary*

**Corresponding author: Márió Gajdács*
Email: mariopharma92@gmail.com

Received: *6 March 2020 / Revised: 22 March 2020 / Accepted: 24 March 2020*

Abstract
Purpose: *The correlation between the levels of antibiotic use (including self-medication with antibiotics) and the development and spread of resistant bacteria has been highlighted by several publications worldwide. The aim of our present study was to assess the knowledge level and attitudes of patients (general population) towards antibiotics and antimicrobial resistance, in addition to their practices towards the procurement and use of these drugs in the Szeged District of Hungary.*
Materials/methods: *A cross-sectional, questionnaire-based pilot study was performed among patients aged 18 years or older in the Szeged District. The study population comprised of adult patients attending their general practitioner's (GP) offices. Data collection for the survey was running between January 2016 and January 2018. Sample size for the adult population of the Szeged District was calculated by using the Raosoft sample size calculator.*
Results: *Responses from n=109 were included in the final analysis. The median age of the respondents was 51 years (50.8±17.8 years, range: 19-93). The majority of respondents were from the seat of the district and the county (Szeged; n=62; 56.9%). 53.7% (n=59) reported having a chronic illness which requires medical attention/pharmacotherapy. Almost one-third (32.1%) has taken these drugs during the last 12 months. 90.5% (n=99) of respondents has obtained their last course of ABs through a medical prescription. The average number of correct answers overall were 2.11±1.16; highest level of education ($p<0.001$) and reported use of antibiotics for inappropriate indications (e.g., sore throat, cold, flu, fever) showed significant associated in the results of knowledge-based questions ($p=0.03$).*
Conclusions: *As the number of available antibiotics is dwindling, one of the most important steps to preserve the efficacy of existing drugs is the use of educational campaigns in an attempt to augment the health behaviour of patients. Higher education levels were associated with better knowledge and attitudes, in addition, the majority of respondents were not aware of the differences between bacterial and viral infections and their treatment. This study has built on existing research and generated data which may be used for the designing and implementation of awareness campaigns, based on the needs of the local community.*

Keywords: *antibacterial agent, drug resistance, public, knowledge, attitude, questionnaire, Eurobarometer*

1. Introduction

Antimicrobial resistance (AMR) is one of the most relevant concerns of modern medicine worldwide: these drugs, considered as *panacea* for the treatment of previously deadly infections are losing their effectiveness, resulting in increased mortality rate and decreased quality of life in the affected patient population [1,2]. The withdrawal of major pharmaceutical companies from the field of antimicrobial R&D means that healthcare professionals have to make due with the drugs that are already available [3,4]. In fact, one of the main requirements for even the "Health for all by 2000" (World Health Organization, 1981) was the availability of effective antibiotics: therefore, one of the main setbacks of this program was the rapid global emergence of AMR [5,6]. The situation of antibiotic availability has not improved substantially since the beginning of the 21st century; in a current report from the WHO, the lack of novel antibiotic has once again been cited as a significant threat to medicine [7]. Infections caused by resistant bacterial pathogens are also sources of significant financial losses (both corresponding to increased expenses for the healthcare infrastructure and loss of profits from decreased productivity): in the United States, these financial losses have been estimated to be around 50-60 billion US dollars, while in the European Union, these figures may go up as high as 1.5 billion euros [1,8].

Hungary ranks low in the total consumption of antibiotics (ABs) in Europe, both in the community and in hospital settings (with a consumption of

DOI: 10.33892/aph.2020.90.5-14

15.4 and 1.18 defined daily doses [DDD] per 1000 inhabitants per day, respectively, based on the latest data of the ECDC [EU/EEA average: 21.9 and 2.06 DDD per 1000 inhabitants per day, respectively], however, the country is a leader in the consumption of broad-spectrum agents (predominantly fluoroquinolones) instead of narrow-spectrum drugs [9,10]. The correlation between the levels of AB use (including self-medication with ABs) and the development and spread of resistant bacteria has been highlighted by several publications worldwide [11]. Although the relationship is complex, the increased utilization of ABs drives increases in resistance levels; therefore, the prudent use of these agents is of utmost importance [12]. In addition to the responsibility of various healthcare professionals (HCPs) (i.e. the compliance of prescribing physicians, pharmacists, nurses with national and international guidelines and regulations, restriction of non-prescription AB sales, proper instructions given by HCPs [13-17]), the populations' knowledge and attitudes on AB use and resistance has been shown to significantly influence antibiotic consumption worldwide [18].

Apart from the knowledge of the general population, the social aspects of AMR and improper antibiotic use are increasingly being recognized as an important facet of this issue: the insufficient social awareness of antibiotic resistance may lead to exaggerated expectations for AB prescriptions from the public; in addition, the socio-economic status, education level, residential background, cultural factors, vulnerable population status, desperate need to maintain employment despite the presence of symptoms of bacterial diseases or discrimination may all influence behavioural biases, health literacy and health-related decision-making processes in the general population [19,20]. These issues are usually addressed via awareness-raising and educational campaigns, both aimed at HCPs and the public. Among the campaigns with the largest impact, the European Antibiotic Awareness Day (EAAD; 18th of November) organized by the European Centers for Disease Control and Prevention (ECDC) and the World Antibiotic Awareness Week (18-24th of November in 2019) by the WHO are among the most notable.

Preceding research on the treatment-seeking behaviors and attitudes of the European population has prompted our research interest to investigate current trends in our local setting [21]. The aim of our present study was to assess the knowledge level and attitudes of patients (general population) towards AB and AMR, in addition to their practices towards the procurement and use of these drugs in the Szeged District of Hungary.

2. Materials and methods

2.1. Sample size and study location

A cross-sectional, questionnaire-based pilot study was performed among patients aged 18 years or older in the Szeged District. The Szeged District is located in Csongrád County, in the Southern Great Plain of Hungary; with an area of ~741 km^2, it is the second largest district in the county, containing thirteen inhabited places (ten villages, one large village and two cities). The population of the county is around 204,000 people, as per most recent census data (population density: 276/km^2).

The study population comprised of adult patients attending their general practitioner's (GP) offices in the District. Sample size for the adult population of the Szeged District was calculated by using the Raosoft sample size calculator [22], based on the formula below (1): population was N=169,300 (83% of the total population is aged 18 or older, as per most recent census data), x was confidence interval of 95%, E was the a margin of error set at 5% and the expected response rate set at 50%, based on the results of the Special Eurobarometer 407 [21]. The minimum sample size of n=103 (n=94 with an added contingency of 10% for non-responders and inappropriate responses) was set for the completion of this pilot survey.

$$n=N\frac{x}{(N-1)E2+x} \quad (1)$$

A total of 218 patients in five GPs' offices throughout the Szeged District were approached with our questionnaire, out of which 113 chose to participate in our survey (corresponding to a response rate of 51.9%, females were more inclined to participate). Four respondents (1.5%) were excluded due to incompletely filled out questionnaires; therefore n=109 were included in the final analysis.

2.2. Structure of the questionnaire

A literature review of similar surveys was conducted in the PubMed/MEDLINE database in order to identify potential questions for the development of the instrument in this study; in addition, some of the questions were based on the questions

of the Eurobarometer Survey 407 [21]. The questionnaire was adapted in accordance with local population characteristics and the questionnaires were developed in Hungarian language. The questionnaire was then reviewed and assessed by three faculty members (with extensive experience in survey research), a GP, a community pharmacist and a public health specialist for content and face validation and to ensure the clarity and ease of use during the survey. Minor modifications (rewording, reformatting or reordering of questions) were performed based on the comments of the evaluation panel.

The final structured questionnaire consisted of 33 questions covering three major areas: *Part I.* demographic characteristics and health status (nine items: age, sex, highest level of education, place of residence, employment status, subjective evaluation of health status, existence of chronic illnesses, reason for present GP-visit); *Part II.*: questions about the use and the procurement of ABs and the roles of HCPs in the eyes of the respondents (fourteen items); this section included single-choice, multiple-choice and open-ended questions, respectively. In addition, some of the questions had follow-up questions, therefore respondents were asked to fill out or skip specific questions depending on their answers; *Part III.*: questions on the knowledge and attitudes of the patients (five-five items each), adopted from the Eurobarometer survey: at the statements evaluating the respondents' knowledge on ABs, they had the option of choosing between 'True', 'False', and 'I don't know/Unsure about the answer', while during statements aiming to measure the attitude of respondents, a five-point Likert-scale ranging from "Strongly agree" to "Strongly disagree" was used to record the responses of the participants. Questions **QK1-QK4** (see *Table 3*) were used to create a composite knowledge-score (ranging between 0-4 for each correct statement) to allow for comparison with the results of the Eurobarometer surveys.

2.3. Data collection, statistical analysis

Data collection for the survey was running between January 2016 and January 2018. Prior to participating in the survey, the nature and purpose of the study was explained to the patients, including the data collection methods and that participation was voluntary. Participants were informed that the data collection, processing and analysis are anonymous. Data collectors also made sure that the patients did not participate in the Eurobarometers regarding ABs in parallel (Special Eurobarometer 445: field work between April-May 2016; Flash Eurobarometer 444: field work between September-October 2016, respectively) [23]. No renumeration or gifts were given to participants to facilitate them to take part in the survey.

All questionnaires were checked manually and questionnaires with >90% completion were included in the analysis. All the completed questionnaires were entered into Epi-data version 3.1 and the data was exported to SPSS (Statistical Package for the Social Sciences) Statistics version 23.0 (IBM; Chicago, IL, USA) for data analysis. Descriptive statistics were used to analyze qualitative variables, while quantitative variables were summarized using mean ± standard deviation (±SD). Univariate analysis was performed using Pearsons's Chi-squared tests or Fisher's exact tests when comparing proportions, while Student's t-tests were utilized to assess the association between numerical values. All statistical tests were two-tailed, and results were considered to be statistically significant if $p<0.05$.

2.4. Ethical approval

The survey was conducted in accordance with the Declaration of Helsinki and national and institutional ethical standards. Ethical approval for the study protocol was obtained from the Regional and Institutional Human Medical Biological Research Ethics Committee of the Szent-Györgyi Albert Clinical Centre, University of Szeged (registration number: 3688).

3. Results

3.1. Demographic characteristics, health status of respondents

The socio-demographic characteristics of the respondents are presented in *Table 1*. The median age of the respondents was 51 years (50.8±17.8 years, range: 19-93). The majority of respondents were from the seat of the district and the county (Szeged; n=62; 56.9%), while other respondents were from villages (43.1%; Domaszék n=41, Szatymaz n=2, Deszk, Kistelek, Kiskundorozsma, Zákányszék n=1, respectively). Among the respondents, females (60.6%) and patients with the secondary-level education (61.5%) were represented in higher numbers.

Table 1 *Demographic characteristics of the participants*

Characteristics	n (%)
Gender	
Female	66 (60.6%)
Male	43 (39.4%)
Age	
18-35 years	27 (24.4%)
36-64 years	50 (45.8%)
65 years or older	32 (29.8%)
Place of residence	
Urban	62 (56.9%)
Provincial	47 (43.1%)
Level of education	
Elementary school	12 (11.0%)
Trade school	28 (25.7%)
Secondary school	18 (16.5%)
Grammar school	21 (19.3%)
College/University	30 (27.5%)
Employment status	
Employed	50 (45.9%)
Unemployed/Looking for a job	6 (5.5%)
Pensioner	38 (34.9%)
Rehabilitation	2 (1.8%)
Disability pension	3 (2.7%)
Childcare benefit/allowance	3 (2.7%)
Student	7 (6.5%)

The respondents evaluated their current health status as bad in 9.3% of cases (2.8% and 6.5% for *very bad* and *bad*, respectively), while 41.7% responded with *moderate* and 51.9% with good (46.3% and 5.4% for *good* and *very good*, respectively) 53.7% (n=59) reported having a chronic illness which requires medical attention/pharmacotherapy (or has required therapy in the last 12 months): 51.7% of these patients reported one chronic disease, while 31.2%, 13.7% and 3.4% reported suffering from two, three and four chronic ailments respectively. The self-reported chronic illnesses were diseases of the cardiovascular system (30.4%), diabetes (type I and II; 21.8%), diseases of the locomotor system (16.3%), endocrine system (7.6%), pulmonary system (7.6%), kidney disease (5.4%), gastrointestinal system (5.4%), autoimmune disorders (2.2%), psychiatric disorders (2.2%) and malignant illness (1.1%). A significant association was found between the self-reported health status and the presence of chronic illnesses ($p<0.0001$), but not between age and chronic illnesses ($p>0.05$).

36.0% of respondents have visited the GPs' office to procure a prescription for themselves or their family members, 31.3% due to an acute illness, 19.6% were on their follow-up visit, 5.5% came for the administration of a medicine or vaccine, 4.8% visited for a routine check-up (for employment purposes or for driving licenses), while 2.8% came for a referral letter to visit a specialist.

3.2. Antibiotic use, sources of antibiotics

80.7% (n=88) of respondents stated that they visit the GPs offices 0-2 times a year due to an infectious disease, while 14.7% (n=16) reported 3-4 and 4.6% (n=5) reported more, than 4 occasions, respectively. Patients were surveyed on the last time they took ABs (results are presented in *Table 2*): almost one-third (32.1%) has taken these drugs during the last 12 months. 90.5% (n=99) of respondents has obtained their last course of ABs through a medical prescription, 1.9% (n=2) had it administered by a medical practitioner, 4.8% (n=5) used leftover drugs from a previous course of therapy, while 2.8% (n=3) obtained it from a pharmacy, without a medical prescription.

By their own admission, 31.6% of respondents stated that the last time they took ABs was to treat sore throat, while 14.0% has taken them for a urinary tract infection, 13.2% to treat a cold, 7.4% for bronchitis, 6.6% to treat a flu or a persistent cough, 5.1% for pneumonia, 4.4% took it prophylactically after surgery, 2.9% took them after a dental procedure, 2.2% took them due to a skin and soft tissue infection or to treat diarrhoea and 1.5% took ABs to alleviate fever.

Patients were asked about their sources of information on ABs (multiple answers were allowed): medical doctors were perceived as the most trustworthy sources (70.6%), followed by community pharmacists (23.9%), the Internet (20.2%), previous secondary school/university education (19.3%), television and/or radio (18.3%), books, newspapers or information pamphlets (17.4%), family members (10.1%), friends or acquaintances (7.3%), other

Table 2 *Responses to the question 'When was the last time you took antibiotics?'*

Last use of antibiotics	n (%)
I have never taken antibiotics.	4 (3.7)
More than 5 years ago.	20 (18.3)
1-5 years ago.	29 (26.6)
In the last 12 months.	35 (32.1)
In the last month.	10 (9.2)
I am currently taking antibiotics.	5 (4.6)
I don't know/I don't remember.	6 (5.5)

Table 3 *Statements measuring respondents' knowledge on ABs*

	True n (%)	False n (%)	Unsure of the answer n (%)
QK1 *Antibiotics are effective against viruses.* ***(Correct answer: False)***	**56 (51.4%)**	**35 (32.1%)**	**18 (16.5%)**
QK2 *Antibiotics are effective therapy for the common cold and the flu.* ***(Correct answer: False)***	**50 (46.3)**	**43 (39.8%)**	**15 (13.9%)**
QK3 *Unnecessary and inappropriate (in dose or duration) use of antibiotics makes them become ineffective.* ***(Correct answer: True)***	**86 (78.9%)**	**7 (6.4%)**	**16 (14.7%)**
QK4 *Taking antibiotics often has side-effects such as diarrhea.* ***(Correct answer: True)***	**74 (65.1%)**	**17 (15.6%)**	**21 (19.3%)**
QK5 *When my symptoms are gone, I may stop taking the antibiotic safely.* ***(Correct answer: False)***	**38 (34.8%)**	**65 (59.6%)**	**6 (5.6%)**

healthcare-professionals, e.g., pharmacy assistants, nurses, health-promotion specialists (2.8%) and other sources (e.g. medicines information leaflet; 1.8%). There were no significant association between age or gender and the reported source of information ($p>0.05$). One third of patients (34.9%; n=38) have recalled receiving information about not taking antibiotics unnecessarily: most of these respondents got this information from their physicians (55.9%), in addition, community pharmacists, family members (11.8%, respectively), the Internet (8.8%), friends or acquaintances and the Internet (5.9%) were also identified. 88.2% (n=30) of these patients chose to take this advice seriously. Older patients recalled receiving information on this topic much less frequently ($p=0.021$).

In addition, patients were asked about their adherence to the medical advice from their respective HCPs: 65.1% stated that they always and completely follow the instructions of the physicians, while 33.9% stated that they generally take these instructions into account; complete adherence to the instructions of the community pharmacists was reported in 45.5%, while generally good adherence in 47.5% of respondents. Only a minority of respondents (0.9% regarding physicians' and 7.1% for pharmacists' instructions) stated that they generally do not follow the instructions of their respective healthcare-providers. 73.1% (n=79) considered the purchase of additional medications or adjuvants (e.g., probiotics) with antibiotics if the pharmacist recommends it. 4.6% (n=5) of patients reported trying to obtain antibiotics from a community pharmacist without a prescription, bypassing their physicians; 1.8% (n=2) of respondents successfully obtained these drugs from a pharmacy. Additionally, 17.8% agreed (5.9% , 7.9%), that they would be able to source antibiotics without a medical prescription, if there were a need for it (53.5% and 22.8% with this statement, while 9.9% was of the answer). This was more prevalent in respondents from Szeged ($p=0.023$), than from respondents from surrounding villages.

3.3. Knowledge and attitude about antibiotics, socio-demographic and key variable analysis

The responses for the knowledge-based questions, adapted from the Eurobarometer survey are presented in *Table 3*. 18.3% (n=20) of respondents gave correct answers to all relevant questions (**QK1-QK4**), while 7.3% (n=8) had zero correct answers; the highest number of respondents could answer two questions (43.1%; n=47) correctly. The average number of correct answers overall were 2.11±1.16; there were no statistically significant differences among the results of respondents from Szeged (2.27±1.10) and respondents from the surrounding villages (1.89±1.19; $p>0.05$). Similarly, no correlation was found between the number of correct answers and age, current health status, presence/absence of chronic illness, employment status or number of GP visits per year ($p>0.05$). There was, however, association found with the highest level of education ($p<0.001$); the same association was also found when considering the number of correct answers to **QK5** ($p=0.029$). In addition, respondents who reported to use antibiotics for inappropriate indications (e.g., sore throat, cold, flu, fever) had worse results in the knowledge-based questions (**QK1-4** $p=0.03$; **QK5** $p=0.047$).

The respondents' beliefs regarding ABs and prevention (questions **QA1-5**) are summarized in *Table 4*. Of note, 75% of respondents believe that ABs are medicines of special importance (**QA1**); there was no correlation between this positive

Table 4 *Patients' attitudes towards antibiotics and preventative measures to avoid contracting infectious diseases*

Statements	SD	D	U/DK n (%)	A	SA
	Disagree n (%)			Agree n (%)	
QA1 *Antibiotics are medicines of special importance.*	5 (4.6%)	11 (10.2%)	11 (10.2%)	37 (34.3%)	44 (40.7%)
QA2 *My knowledge regarding infectious diseases is appropriate.*	3 (2.8%)	20 (18.5%)	15 (13.9%)	48 (44.4%)	22 (20.4%)
QA3 *Personal hygiene and taking care of ourselves have important roles in the prevention of infectious diseases.*	1 (0.9%)	0 (0%)	0 (0%)	26 (23.9%)	82 (75.2%)
QA4 *Vaccines are an important means of preventing infectious diseases.*	8 (7.4%)	6 (5.6%)	10 (9.3%)	31 (28.7%)	53 (49.1%)
QA5 *The media devotes enough energy to disseminate information on infectious diseases.*	16 (15.0%)	26 (24.3%)	17 (15.9%)	29 (27.1%)	19 (17.8%)

SD: strongly disagree, D: disagree, U/DK: I don't know/Unsure, A: agree, SA: strongly agree

statement and the participant's age, gender or the number of correct answers (see previous section), however, participants with higher levels of education ($p=0.048$) and those, who reportedly visit the GP's office more frequently due to infections ailments ($p=0.021$) were more likely to have such beliefs. Only 64.8% of the respondents regarded their knowledge about infectious diseases as appropriate (**QA2**). Interestingly, women were more satisfied with their knowledge-level ($p<0.001$), while satisfaction-level and the number of correct answers showed no association ($p>0.05$). In a similar fashion, women were more likely to be dissatisfied with the involvement of the media in disseminating relevant information on infectious diseases to the public (**QA5**; $p=0.024$); although 44.9% agreed and 39.3% of the patients disagreed with the statement. All respondents agreed on the role of personal hygiene and personal care (100%) in infectious disease-prevention, however, the role of vaccines in this regard was clear to only 77.8% of participants (**QA3** and **QA4**, respectively).

4. Discussion

In the present study, the knowledge level and attitudes of patients towards ABs and antibiotic resistance, in addition to their drug utilization practices were assessed in the Szeged District of Hungary. We have found that around 32% of respondents have taken ABs in the last 12 months, mainly for a sore throat. They identified healthcare professionals as the most trustworthy sources of information, moreover they generally follow their advices; while the Internet also emerged as an important information source, verifying the results of previous reports [24]. Just minority (4.8%) used leftover ABs for their last course, and 2.8% accessed to non-prescribed ABs from a pharmacy. This latter is in line with the result of a European study, where 1-9% of the population was identified generally to obtain ABs without a prescription from a pharmacy [25]. Regarding the knowledge level of the respondents, we have found that most patients were aware of the emergence of bacterial resistance and the potential adverse events that may occur during AB use, however, many respondents identified colds, the flu and viral infections in general as indications for AB therapy. Based on our results, correlation was found between the highest level of education, AB-related knowledge and the appreciation of the role of ABs in healthcare, which is line with other reports available in the literature [26,27].

The methodology and instrument used for this survey was based and adapted from the Eurobarometer survey on AMR by the European Commission, one of the two most important international studies, the Special Eurobarometer reports (Flash EBM 444 [28] in 2016, EBM 445 [23] in 2016 and EBM 478 in 2018 [39], respectively) commissioned by the European Union, and the 'Antibiotic Resistance: Multi-country awareness survey' for non-EU countries, performed by the WHO [30]. The highlights of the abovementioned reports are summarized in *Table 5*, serving as a contrast to the results of the present study. The average number of correct answers in the study region (2.1) was in line with previous reports of the Hungarian national average (in the EBMs 338, 407 and 445), however, in the last EBM, both the European overall average and the national average of Hungary has increased (2.6 and 2.3, respectively) [21,23,28-30].

Table 5 *Summary of the findings of the Special Eurobarometer reports (EU) and the 'Antibiotic Resistance: Multi-country awareness survey' (WHO) on AB-related knowledge and AB utilization [21,23,28-30].*

Survey (Year)	Antibiotics in the last 12 months (EU)	Source of antibiotics (EU)	Antibiotics taken for the flu	Antibiotics taken to treat a cold	Average number of correct answers (EU)	Antibiotics are effective against the cold and flu	Antibiotics are effetive against viruses	All answers (n=4) are correct
EBM 407 (2013) [21]	35%; highest in Malta (48%), lowest in Sweden (24%)	From a medical prescription/ pharmacy: 95%; 3% from a pharmacy, without a prescription; 2% leftover from home	18%	13%	2.4 (Hungary: 2.1); highest in Sweden (3.1), lowest in Romania (1.5)	41%	49%	22%
EBM 445 (2016) [23]	34%; highest in Malta (48%), lowest in Sweden (18%)	From a medical prescription/ pharmacy: 93%; 4% from a pharmacy, without a prescription; 3% leftover from home	16%	11%	2.5 (Hungary: 2.2); highest in Finland (3.1), lowest in Italy (1.9)	44%	43%	24%
EBM 478 (2018) [29]	32%; highest in Italy (47%), lowest in Sweden (20%)	From a medical prescription/ pharmacy: 93%; 3% from a pharmacy, without a prescription; 4% leftover from home	12%	8%	2.6 (Hungary: 2.3); highest in Finland and Sweden (3.1), lowest in Romania and Latvia (2.1)	48%	28%	25%
WHO Multi-country survey (2015) [30]	35%; higher in lower income countries (42%), lower in higher income countries (29%)	From a medical prescription/ pharmacy: 93%; 2% from a pharmacy, without a prescription; 2% leftover from home; 3% from a friend or family member, 1% from the Internet	-	-	-	64%	-	-

By the recommendations of the European Surveillance of Antimicrobial Consumption Network (ESAC-Net), AB use in adults is only indicated in around 0-30% of cases in upper respiratory tract infections, acute tonsillitis, acute or chronic sinusitis, acute otitis media, acute bronchitis or bronchiolitis, while for acute cystitis and pneumonia, this ratio is 80-100% [10,11]. Several national and international studies have described gaps in public knowledge and issues regarding inappropriate AB use [11,18,31-34], characterized by their procurement through non-prescription (predominantly

from pharmacies) and non-medical (leftover drugs, drugs from family members or friends) sources. These studies have all highlighted the role of self-medication of antibiotics as a minor (0.5-8%), but still significant portion of AB consumption. In addition, the ignorance (risk-taking behaviour) or lack of understanding towards in ineffectiveness of ABs against viral infections (and the common cold) was also identified in a plethora of publications: many people identify all disease-causing pathogens as "germs", not distinguishing their biological characteristics and the subsequent therapeutic approach needed to treat them [11,18,31-34].

Educational campaigns and behavioural AMR strategies are essential to address both the knowledge-based and social aspects of inappropriate AB use in the public, including the lowering of expectations for AB prescriptions, and the highlighting of the dangers associated with AMR [35]. On the other hand, healthcare professionals' responsibility on the matter also has to be highlighted: as the doctor-patient and pharmacist-patient relationship is unbalanced (from the standpoint of medical information), patients will adhere to the advice received (also highlighted by the results of this study) and feed off of the inappropriate behaviors and attitudes of the respective healthcare professionals [17,36,37]. Thus, it may be concluded that without structural changes in the healthcare infrastructure of a relevant country, true change cannot be attained [38].

Some limitations of this study must be acknowledged (which were also present in the standardized Eurobarometer instrument): i) some of the questions rely on the respondents' memory, which may lead to discrepancies or bias; ii) the principal assumption of the study is that the knowledge level and attitude of patients will indefinitely determine their practices towards ABs and the practices of the respondents were not measured directly; iii) the presence of social desirability bias in some of the questions; iv) geographical limitations (the study may only represent the patients of the Szeged District).

5. Conclusions

As the number of available antibiotics is dwindling, one of the most important steps to preserve the efficacy of existing drugs is the use of educational campaigns in an attempt to augment the health behaviour of patients. Our paper aimed at the assessing and understanding of AMR-related general population behaviour in the Szeged District of Hungary, in a descriptive antibiotic-related questionnaire, based on the methodology of the Special Eurobarometer reports of the European Commission. As a highlight to our study, higher education levels were associated with better knowledge and attitudes, in addition, the majority of respondents were not aware of the differences between bacterial and viral infections and their treatment. This study has built on existing research and generated data which may be used for the designing and implementation of awareness campaigns, based on the needs of the local community.

Author Contributions

M.G. and A.S. conceived and designed the study, performed data collection and analysis, wrote and revised the full paper. E.P. supervised the completion of the study wrote and revised the full paper**.**

Funding

This research received no external funding.

Acknowledgments

The authors would like to express their gratitude to the patients who participated in this study. The authors would like to acknowledge the help of Gábor Oszlánczi MD PhD, Emese Petra Balogh MD PhD, Judit Baranyai MD, Nándor Pördi MD and Gábor Kőrösi MD in reaching the participants of this study. Part of this study was presented at the 28th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID).

Conflicts of Interest

The author declares no conflicts of interest, monetary or otherwise.

References

1. Cassini A, Högberg DL, Plachouras D, Qattrocchi A, Hoxha A, Simonsen G, Colomb-Cotinat M, Kretzschmar ME, Devleesschauwer B, Cecchini M, Ouakrim DA, Oliviera CT, Struelens MJ, Suetens C, Monnet DL, Burden of AMR Collaborative Group. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect Dis 2019; 19: 56-66. https://doi.org/10.1016/S1473-3099(18)30605-4

2. Gajdács M, Zupkó I. Az antibiotikum rezisztencia krízis: lépést tudnak-e tartani a gyógyszerfejlesztő cégek? [The antibiotic resistance crisis: can pharmaceutical companies keep up with the pace?] (article in Hungarian). Gyógyszerészet 2019; 63: 736-742.
3. Spellberg B. The future of antibiotics. Crit. Care. 2014; 18: 228. https://doi.org/10.1186/cc13948
4. Gajdács M. The Concept of an Ideal Antibiotic: Implications for Drug Design. Molecules 2019; 24: e892. https://doi.org/10.3390/molecules24050892
5. WHO Global Strategy for Health for All by the Year 2000. [Internet] [cited 7 January 2020]. Available from: https://iris.wpro.who.int/bitstream/handle/10665.1/6967/WPR_RC032_GlobalStrategy_1981_en.pdf
6. WHO Global action plan on antimicrobial resistance. [Internet] [cited 7 January 2020]. Available from: https://apps.who.int/iris/bitstream/handle/10665/193736/9789241509763_eng.pdf?sequence=1
7. Adeel Aslam, Mário Gajdács, Che Suraya Zin, Norny Syafinaz Binti Abd Rahman, Syed Imran Ahmed, Shazia Jamshed: Public Awareness and Practices towards Self-Medication with Antibiotics among the Malaysian Population. A Development of Questionnaire and Pilot-Testing. Antibiotics 2020; 9: e97. https://doi.org/10.3390/antibiotics9020097
8. Founou RC, Founou LL, Essack SY. Clinical and economic impact of antibiotic resistance in developing countries: A systematic review and meta-analysis. PLoS One 2017; 12: e0189621. https://doi.org/10.1371/journal.pone.0189621
9. ESAC-Net. Antimicrobial consumption in the EU/EEA Annual epidemiological report for 2018. [Internet] [cited 7 January 2020]. https://www.ecdc.europa.eu/sites/default/files/documents/Antimicrobial-consumption-EU-EEA.pdf
10. Matuz M, Benkő R, Dóró P, Hajdú E, Nagy E, Monnet DL, Soós G. Regional variations in community consumption of antibiotics in Hungary, 1996-2003. Br J Clin Pharmacol 2006; 61: 96-100. https://doi.org/10.1111/j.1365-2125.2005.02525.x
11. Abduelkarem AR, Othman AM, Abuelkhair ZM, Ghazal MM, Alzouobi SB, Zowalaty MEE. Prevalence Of Self-Medication With Antibiotics Among Residents In United Arab Emirates. Infect Drug Res 2019; 12: 3445-3453. https://doi.org/10.2147/IDR.S224720
12. Barbosa TM, Levy SB. The impact of antibiotic use on resistance development and persistence. Drug Res Updat 2000; 3: 303-311. https://doi.org/10.1054/drup.2000.0167
13. Gajdács M, Szabó A. Orvosok antibiotikum felhasználással és rezisztenciával kapcsolatos véleményének vizsgálata Magyarország délkeleti részén. [Physicians' opinions towards antibiotic use and resistance in the southeastern region of Hungary] (article in Hungarian). Orv Hetil, accepted.
14. Gajdács M, Paulik E, Szabó A. Közforgalomban dolgozó gyógyszerészek antibiotikum felhasználással és rezisztenciával kapcsolatos véleménye [The opinions of community pharmacists related to antibiotic use and resistance] (article in Hungarian). Acta Pharm Hung 2018; 88: 249-252.
15. Jamshed S, Padzil F, Shamsudin SH, Bux SH, Jamaluddin AA, Bhagavathula AS, Azhar S, Hassali MA. Antibiotic Stewardship in Community Pharmacies: A Scoping Review. Pharmacy 2018; 6: e92. https://doi.org/10.3390/pharmacy6030092
16. WHO Competency Framework for health workers' education and training on antimicrobial resistance. [Internet] [cited 7 January 2020]. Available from: http://apps.who.int/medicinedocs/documents/s23443en/s23443en.pdf
17. ECDC. Survey of healthcare workers' knowledge, attitudes and behaviours on antibiotics, antibiotic use and antibiotic resistance in the EU/EEA. [Internet] [cited 7 January 2020]. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/survey-of-healthcare-workers-knowledge-attitudes-behaviours-on-antibiotics.pdf
18. Zajmi D, Berisha M, Begolli I, Hoxha R, Mehmeti R, Mulliqi-Osmani G, Kurti A, Loku A, Raka L. Public knowledge, attitudes and practices regarding antibiotic use in Kosovo. Pharmacy Practice 2017; 15: 827. https://doi.org/10.18549/PharmPract.2017.01.827
19. Macfarlane J, Holmes W, Macfarlane R, Britten N. Influence of patients' expectations on antibiotic BMJ 1997; 315: 1211-14. https://doi.org/10.1136/bmj.315.7117.1211
20. Mangione-Smith R, McGlynn EA, Elliott MN, Krogstad P, Brook RH. The relationship between perceived parental expectations and pediatrician antimicrobial prescribing behavior. Pediatrics 1999; 103: 711-18. https://doi.org/10.1542/peds.103.4.711
21. European Commission. Special Eurobarometer 407: Antimicrobial Resistance. [Internet] [cited 7 January 2020]. Available from: https://ec.europa.eu/commfrontoffice/publicopinion/archives/ebs/ebs_407_en.pdf
22. Raosoft Sample Size Calculator. [Internet] [cited 7 January 2020]. Available from: http://www.raosoft.com/samplesize.html
23. European Commission. Special Eurobarometer 445: Antimicrobial Resistance. [Internet] [cited 7 January 2020]. Available from: https://ec.europa.eu/health/amr/sites/amr/files/eb445_amr_generalreport_en.pdf
24. Mandle G, Kostkova P, Weinberg J. Bugs and drugs on the Web: changes in knowledge of users of a web-based education resource on antibiotic prescribing. J Antimicrob Chemother 2009; 63: 221-223. https://doi.org/10.1093/jac/dkn438
25. Safrany N, Monnet DL. Antibiotics obtained without a prescription in Europe. Lancet Infect Dis. 2012; 12: P182-P183. https://doi.org/10.1016/S1473-3099(12)70017-8
26. de Bont EGPM, Alink M, Falkenberg FCJ, Dinant GJ, Cals JWL. Patient information leaflets to reduce antibiotic use and reconsultation rates in general practice: a systematic review. BMJ Open 2015; 5: e007612. https://doi.org/10.1136/bmjopen-2015-007612
27. Burstein VR, Trajano RP, Kravitz RL, Bell RA, Vora D, May LS. Communication interventions to promote the public's awareness of antibiotics: a systematic review. BMC Public Health 2019; 19: e899. https://doi.org/10.1186/s12889-019-7258-3
28. European Commission. Flash Eurobarometer 444: Antimicrobial Resistance. [Internet] [cited 7 January 2020]. Available from: https://ec.europa.eu/commfrontoffice/publicopinion/index.cfm/ResultDoc/download/DocumentKy/75687
29. European Commission. Special Eurobarometer 478: Antimicrobial Resistance. [Internet] [cited 7 Janu-

ary 2020]. Available from: http://ec.europa.eu/commfrontoffice/publicopinion/index.cfm/ResultDoc/download/DocumentKy/84386
30. WHO. Antibiotic Resistance: multi-country public awareness survey https://apps.who.int/medicinedocs/documents/s22245en/s22245en.pdf
31. Vallin M, Polyzoi M, Marrone G, Rosales-Klintz S, Tegmark WK, Stalsby LC. Knowledge and Attitudes towards Antibiotic Use and Resistance - A Latent Class Analysis of a Swedish Population-Based Sample. PLoS One 2016; 11: e0152160. https://doi.org/10.1371/journal.pone.0152160
32. Mazinska B, Struzycka I, Hryniewicz W. Surveys of public knowledge and attitudes with regard to antibiotics in Poland: Did the European Antibiotic Awareness Day campaigns change attitudes? PLoS One 2017; 12: e0172146. https://doi.org/10.1371/journal.pone.0172146
33. Ramchurren K, Balakrishna Y, Mahomed S Patients' knowledge, attitudes and practices regarding antibiotic use at a regional hospital in KwaZulu-Natal, South Africa 2017. S Afric J Infect Dis 2018; 33: a146. https://doi.org/10.4102/sajid.v33i5.146
34. Kong LS, Islahudin F, Muthupalaniappen L, Chong WW. Knowledge and Expectations on Antibiotic Use among Older Adults in Malaysia: A Cross-Sectional Survey. Geriatrics 2019; 4: e61. https://doi.org/10.3390/geriatrics4040061
35. Matthew P, Sivaraman S, Chandy S. Communication strategies for improving public awareness on appropriate antibiotic use: Bridging a vital gap for action on antibiotic resistance. J Family Med Prim Care 2019; 8: 1867-1871. https://doi.org/10.4103/jfmpc.jfmpc_263_19
36. Gajdács M, Paulik E, Szabó A Közforgalomban dolgozó gyógyszerészek attitűdvizsgálata a fertőző betegségekkel kapcsolatos szerepkörük kiszélesítésére vonatkozóan Magyarország délkeleti részén [The attitude of community pharmacists towards their widening roles in the prevention and treatment of infectious diseases in the southeast region of Hungary] (article in Hungarian). Gyógyszerészet 2019; 63: 26-30.
37. Rábano-Blanco A, Domínguez-Martins EM, Mosteiro-Miguéns DG, Freire-Garabal M, Novio S. Nursing Students' Knowledge and Awareness of Antibiotic Use, Resistance and Stewardship: A Descriptive Cross-Sectional Study. Antibiotics 2019; 8: e203. https://doi.org/10.3390/antibiotics8040203
38. Malik B, Bhattacharyya S. Antibiotic drug-resistance as a complex system driven by socio-economic growth and antibiotic misuse. Sci Rep 2019; 9: e9788. https://doi.org/10.1038/s41598-019-50846-1

Spearmint (*Mentha spicata* L.) essential oil from tipaza (Algeria): *in vivo* anti-inflammatory and analgesic activities in experimental animal models

SARAH KEHILI [1], MOHAMED NADJIB BOUKHATEM [2*], ASMA BELKADI [3], FAIZA BOULAGHMEN [4], MOHAMED AMINE FERHAT [1], WILLIAM N. SETZER [5,6]

[1] *Laboratory of Research on Bio-Active Products and Valorization of Biomasse, Department of Chemistry, Ecole Normale Superieure, Algiers, Algeria*
[2] *Department of Biology and Cell Physiology, Faculty of Life and Natural Sciences, University – Saad Dahlab – Blida 1, Blida, Algeria*
[3] *Laboratory of Pharmacology and Toxicology, Research and Development Center, SAIDAL Pharmaceuticals, Algiers, Algeria*
[4] *Anatomic Pathology Laboratory, Hospital - Dr Fares Yahia - Kolea, Tipaza, Algeria*
[5] *Department of Chemistry, University of Alabama in Huntsville, Huntsville,USA*
[6] *Aromatic Plant Research Center, Lehi, USA*

*Corresponding Author: *Mohamed Nadjib Boukhatem*
Email: mn.boukhatem@yahoo.fr

Received: *13 March 2020 / Revised: 6 April 2020 / Accepted: 8 April 2020*

Abstract

Introduction: *Although analgesic and nonsteroidal anti-inflammatory drugs (NSAIDs) are usually used to treat a diversity of illnesses, their administration is linked with acute kidney injury and gastrointestinal side effects. The research of new biomolecules and natural products is still needed such as medicinal plants.*

Aims: *The present research was aimed to investigate, for the first time, the anti-inflammatory and anti-nociceptive effects of spearmint essential oil (SEO) in mouse models of acute inflammation and pain.*

Materials and Method: *Chemical analysis of SEO was done by gas chromatography. The anti-inflammatory activity was tested using two models of acute inflammation namely carrageenan-induced paw edema and xylene-induced ear edema. Histological examination of both non-inflamed and inflamed tissues was evaluated. The anti-nociceptive activity was tested using the pain model induced by acetic acid.*

Results: *The main constituent of the SEO was found to be carvone (52.60%). The SEO exhibited a promising anti-inflammatory effect as demonstrated by statistically significant ($p<0.05$) inhibition of paw volume by 77.24% at the dose of 20 µL/kg and 65.87% at the dose of 200 µL/kg. Furthermore, topical administration of the SEO inhibited xylene-induced ear edema in comparison with the control group ($p<0.05$). The higher dose (200 µL /kg) significantly ($p<0.001$) reduced xylene-induced ear edema which was similar to that observed with positive control (ketoprofen). The pathological analysis of the paws and ears revealed that SEO was capable of reducing cellular infiltration and subcutaneous edema. Else, the SEO produced significant anti-nociceptive activity ($p<0.001$) at higher dose by inhibiting spontaneous nociception.*

Conclusion: *These results support the use of SEO in the development of pharmaceuticals for the management of inflammation and pain.*

Keywords: *Spearmint essential oil; Anti-inflammatory effect; Carvone; Histological analysis; anti-nociceptive activity.*

Abbreviations: ANOVA = Analysis of variance; COX-2 = Cyclooxygenase-2; EO = Essential oil; GC-MS = Gas Chromatography-Mass Spectrometry; H&E = Hematoxylin-Eosin; IL= Interleukin; iNOS = Nitric Oxide Synthase; INF-γ = Interferon; IC50 = Median inhibitory concentration; LPS = Lipo-polysaccharide ; LTB4 = Leukotriene B4 ; NIH = National Institute of Health; NIST = National Standard Institute Technology; NSAIDs = Nonsteroidal Anti-Inflammatory Drugs; NV = Neovascularization; *p.o.* = *per os;* PBS = Phosphate-buffered saline; PGE2 = Prostaglandin E2; SEO = Spearmint essential oil; PMN = Polymorphonuclear cells'; RT = Retention Times; SD = Standard Deviation; TNF = Tumor Necrosis Factor.

DOI: 10.33892/aph.2020.90.15-26

1. Introduction

Inflammation is an imperative biological reaction which happens in answer to a varied range of injurious agents such as physical trauma, bacterial infection, chemicals or any additional physical phenomenon. Inflammation is the complex state of hyperemia from blood vessels with resulting warmness, redness, swelling and discomfort [1]. Immune reaction is important for the body to eliminate and remove hazardous pathogens by way of an acute inflammation [2]. Inflammation usually implicates edema and pain at the location of injury or wound due to discharge of many pro-inflammatory chemical mediators along with the escape of liquid from the vascular tissues due to increase in the penetrability of the vessel barriers, tissue damage, infiltration and migration of inflammatory cells and healing [3]. All kinds of pain, whether it is chronic or acute, initiate from inflammation. During inflammation, several pro-inflammatory chemical factors such as interferon (INF-γ), tumor necrosis factor (TNF), cyclooxygenase-2 (COX-2), interleukins (IL-6 and IL-12), and inducible nitric oxide synthase (iNOS) are synthetized and secreted. The receptors involved in inflammation are similarly stimulated in pain [4].

Non-steroidal anti-inflammatory drugs (NSAIDs), such as diclofenac sodium, are among the most frequently recommended medications due to their regular efficiency in the management and treatment of inflammation, pain and rheumatic complaints [5]. Nevertheless, their administration is linked with undesirable effects at the level of digestive area, ranging from gastrointestinal erosions and severe peptic ulcers to more grave problems, such as over bleeding or full damage of epithelial membrane. Other NSAIDs adverse reactions comprise hepatic, renal and cardiovascular undesirable effects which are correlated to the agent as well as the frequency and period of treatment [6]. Then, to overcome the toxicity of NSAIDs, the investigation of new and promising molecules is still required and the natural products such as aromatic and medicinal plants could lead in the development of new anti-inflammatory and analgesic drugs with fewer side effects.

Numerous species of *Mentha* genus are used in phytotherapy as flavor agents and as herbal medicines for several human therapeutic administrations [7]. Spearmint (*Mentha spicata* L.) is generally cultivated in different regions worldwide to commercially produce its aromatic or essential oil (EO) [8]. Spearmint has several therapeutic properties and health benefits and is used in phytomedicine as antifungal, diuretic, carminative and antioxidant agents, and for management of colds and flu, hemorrhoids, respiratory tract problems and stomach ache. Interestingly, spearmint essential oils (SEOs) were recognized as rich in oxygenated monoterpenes and were found to have antimicrobial activity [9,10]. It is also a nontoxic and real therapeutic choice for the cure of chemotherapy-induced emesis and nausea in patients [11].

Although several studies have been reported on the chemical composition, antimicrobial and antioxidant activities of spearmint essential oil *in vitro* [7-10], however only few investigations report its pharmacological effects *in vivo*. Based on the above reflections, the present research reports, for the first time, the chemical composition of essential oil obtained from the leaves and stems of spearmint grown in Algeria as well as the *in vivo* anti-inflammatory and anti-nociceptive effects.

2. Material and methods

2.1. Materials

2.1.1. Extraction of Spearmint essential oil

Spearmint (*Mentha spicata* L.) leaves and stems were collected on june 2016 in the Cherchell region (Tipaza, Algeria). This area is located in the eastern region of Algiers (coordinates 35°52′34″N latitude and 0°17′20″W longitude). SEO was extracted from the aerial part of spearmint with and alembic steam distillation (Extral-Bio company, Chiffa, Blida, Algeria). The whole quantity of fresh plant used was 450 kg, and it was loaded in the still and stacked in layers to allow appropriate delivery of the steam. The process consists of passing water vapor at a high-pressure through an alembic (tank) filled with spearmint. The SEO was stored in a sealed vial, at 4 °C, before use.

2.1.2. Chemicals and drugs

The following drugs and chemicals were used: dimethyl sulfoxide (DMSO), sodium diclofenac, carrageenan, xylene, tween 80, isosaline (0.85%) and phosphate-buffered saline (PBS) solutions were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ketum gel® 2.5% (Ketoprofene 60 g,

Laboratoire Menarini, Barcelona, Spain), Spasfon® 80 mg (Phloroglucinol, Teva Sante, Paris, France), Ketamile® (Ketamine chlorydrate, El Kendi Pharmaceuticals, Algiers, Algeria) and Votrex® 50 mg (sodium diclofenac, Hikma Pharmaceuticals, Jordan) were also used.

2.1.3. Animals

Swiss NMRI male mice (25.28±1.75 g) were got from the animal breeding of SAIDAL pharmaceutical company (Algiers, Algeria). The mice were used in agreement with the guiding principles in the care and use of animals agreed by the Council of the American Physiologic Society. The mice were permitted free access to water and food under conditions of optimum light (12 hours light-dark cycle) with the room temperature maintained at 25±3 °C and relative humidity of 45-60%. In anti-inflammatory and analgesic assays, mice were arbitrarily separated into five groups: control, NSAID, and SEO (200, 20, and 2 μL/kg) groups. All experiments were done between 9:00 a.m. and 3:00 p.m. All labors were done in order to diminish animal suffering.

2.2. Methods

2.2.1. Determination of the chemical composition of spearmint essential oil

The chromatographic analysis of the SEO was done using a Shimadzu gas chromatograph (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector QP-5050A. The GC-MS system was equipped with a Tracsil Meta.X5 column, 95% polydimethylsiloxane and 5% polydiphenylsiloxane (Teknokroma S. Coop. C. Ltd., Barcelona, Spain; 60 m × 0.25 mm × 0.25 μm film thickness). Analysis was done using He as carrier gas at a column flow of 0.3 mL/min with a split ratio of 1:200. The oven temperature program was as follow: (a) 80 °C, 0 min; (b) rate of 4 °C/min from 80 to 210 °C and hold for 1 min; (c) rate of 25 °C/min from 210 to 300 °C and hold for 8 min. The temperatures of the injector and detector were 230 and 300 °C, respectively. All chemical compounds were detected and identified using two different techniques: (1) mass spectra (NIST05 spectral library collection), and (2) comparison of their experimental retention indices (RI) with those of the literature. Only fully identified compounds are reported in this study.

2.2.2. In vivo anti-inflammatory activity

2.2.2.1. Carrageenan-induced paw edema in mice

Carrageenan-induced paw edema in mice is a well-established technique of acute inflammation for evaluation of anti-inflammatory molecules. This method was similar to that reported by Mogosan et al. [12]. Mice (six weeks old) weighing about 24–27 g were randomly divided into five groups of six animals; including a control group (vehicle, isosaline NaCl 0.9%); SEO groups (2, 20 and 200 μL/kg), and NSAIDs group (diclofenac sodium, 50 mg/kg), as the standard drug. The designated groups were orally treated with vehicle, SEO, or NSAIDs 40 minutes before λ-carrageenan injection. For induction of inflammation, the mice received an injection of 100 μL of 1% (w/v) suspension of λ-carrageenan in saline, into the mouse›s plantar side of the left hind paw after 40 min of the oral administration of the sample. The animals were sacrificed 4 hours later. The difference in weight between right untreated and left treated hind paws was calculated and results are expressed as the increase in paw weight (mg). The percentage inhibition of the inflammatory response was calculated by comparison to the negative control and SEO or NSAIDS groups by using this formula:

$$\% \text{ Inhibition of edema} = \left(\frac{(C_t\text{-}C_0)\text{Control} - (C_t\text{-}C_0)\text{Treated}}{(C_t\text{-}C_0)\text{Control}}\right) \times 100$$

Where $(C_t - C_0)$ control is the difference in the weight of paw at 4 hours in control animal, and $(C_t - C_0)$ treated is the difference in the weight of paw at 4 hours in animal treated either with the standard drug or SEO.

At the end of the assay, the mice were euthanized by diethyl ether and the inflamed paws were removed and fixed in 10% formaldehyde solution for histological analysis and examination.

2.2.2.2. Histological examination of mouse paw tissue

To further confirm the inflammatory changes in paw of animals after injection of carrageenan, the mice were sacrificed 4 h after the generation of inflammation. The sub-plantar paw tissue samples were then removed, fixed in 10% neutralized formalin for 24 h, dehydrated with alcohol (ethanol), surrounded in paraffin wax (56 °C) and cut into 5 μm thick sections. Successive sections were stained with haematoxylin and eosin (H&E) stain

to establish the degree of edema and inflammation [13]. The stained sections were examined under a light microscope and the histological modifications were registered with the assistance of a pathologist. The severity of paw tissue inflammation was evaluated by two independent observers blinded to the method protocol.

2.2.2.3. Xylene-induced mice ear edema

Topical inflammation was done using ear edema model according to the method of Boukhatem et al. [13]. Adult Swiss albino mice were arbitrarily divided into five groups, each containing six mice. The acute inflammation was made on the posterior and anterior surfaces of the right ear by the topical application of 20 μL/ear of xylene. The left ear (untreated) was considered as a control. To determine the topical anti-inflammatory effect, SEO diluted in almond oil was applied topically at different doses (200, 20 and 2 μL/kg) 40 minutes before the xylene application. Another group was treated only with vehicle (almond oil) and was considered as a control. Ketoprofen (NSAIDs) was used as a reference drug. Four hours after the application of xylene, the animals were sacrified and two ear punches (6 mm diameter) were taken from from both untreated (left) and treated (right) ears and weighed. The weight difference between the right and left ear disks of the same mice was assessed as the intensity of edema. The anti-inflammatory effect was estimated and expressed as a percentage reduction of edema in treated mice compared with the control and calculated using the following formula:

$$\% \text{ Inhibition of topical edema} = \left(1 - \frac{\Delta Wt}{\Delta Wc}\right) \times 100$$

where ΔWt is the change in weight of ear tissue in the treated mice, and ΔWc the change in weight of ear tissue in the control mice (vehicle).

2.2.2.4. Histological examination of mouse ear tissue

The subsequent inflammatory response was assessed and monitored by estimation of edema formation and by microscopic observation. For the histological study of cutaneous inflammation, two samples of the swollen ears from the control and the SEO treated groups were removed and fixed in 10% formaldehyde for one week. Then, the fixed ear tissues were implanted in paraffin, routinely processed and sectioned at 5 μm using a microtome (LEICA RM, Nussloch, Germany). The slices were mounted on the glass slides, stained with H&E and lastly observed and examined by a pathologist in a blinded way. The ear tissues were observed with a light microscope (Olympus CX41) and graded as minor (+), modest (++), or severe (+++) for inflammation phase. Infiltration and polymorphonuclear (PMN) cells' accumulations and infiltration were also reported [13]. The histological examination was carried out in the laboratory of histopathology, Hospital of Kolea (Tipaza city, Algeria).

2.2.3. In vivo anti-nociceptive activity of SEO using acetic acid-induced writhing test

The anti-nociceptive activity of SEO was investigated using the writhing assay (abdominal constriction test), according to the method of Mogosan et al. [12]. A total number of 30 male mice were divided into five groups. Briefly, mice were treated orally with phloroglucinol (Spasfon 80® mg/kg, i.p.) which is considered as a standard analgesic drug. Also, SEO and vehicle (isosaline, NaCl 0.9%) were administrated orally 30 min. After that, intra-peritoneal injection of acetic acid (0.6%) was done for the induction of abdominal contractions. The number of abdominal contractions or writhes were counted for each group of mice starting from one minute after the injection of acetic acid up to 10 minutes and expressed as percent protection. The anti-nociceptive activity was estimated as the percentage of inhibition of abdominal contractions between the control and the treated groups (SEO or standard drug) using the following formula:

$$\text{Protection (\%)} = \left(\frac{N_c - N_t}{N_c}\right) \times 100$$

Where N_c is number of writhing in control, and N_t is the number of writhing in test mice.

2.2.4. Statistical analysis

Data obtained in our research was presented as mean ± SD where each value represents a minimum of 6 mice. One way analysis of variance (ANOVA) was done to evaluate the variability among groups. Significant differences among groups were calculated using Tukey's multiple comparison tests in which the results were compared with that of control group. The results were considered statistically significant at $p<0.05$. XLSTAT software 2014 for Windows (Addinsoft, Paris, France) was used for all statistical analysis.

3. Results and discussion

3.1. Chemical composition of spearmint essential oil using GC-MS analysis

The EO was extracted by steam distillation from the dried aerial parts of spearmint from the Cherchell region (Tipaza, Algeria), and was analyzed by GC-MS (*Figure 1* shows a representative chromatogram). The SEO showed a diverse composition with 19 constituents reported in *Table I* The most abundant chemical components were oxygenated monoterpenes 64.7%, followed by monoterpene hydrocarbons (28.84%), and sesquiterpene hydrocarbons (6.46%). The main constituents were carvone (52.60%) and limonene (24.99%), followed by 1.8-cineole (7.22%) and *trans*-caryophyllene (3.38%). Other chemical compounds were detected but were less than 3%.

The EO of this Algerian variety of mint can be ascribed to the carvone/limonene chemotype. Actually, there is great variation in the chemical composition of spearmint, cultivated as well as endemic or wild, around the world. Remarkably, our SEO seems to be richer with some main compounds such as eucalyptol (1.8-cineole) and oxygenated monoterpenes. A review of the literature accessible on this theme shows that several articles have previously been published on SEOs chemical composition [10,11]. In addition, our findings about most major chemical constituents of SEO collected from the center part of Algeria are in agreement with previous reports [8,9]. Govindarajan et al. [14] studied the chemical composition of SEO obtained from

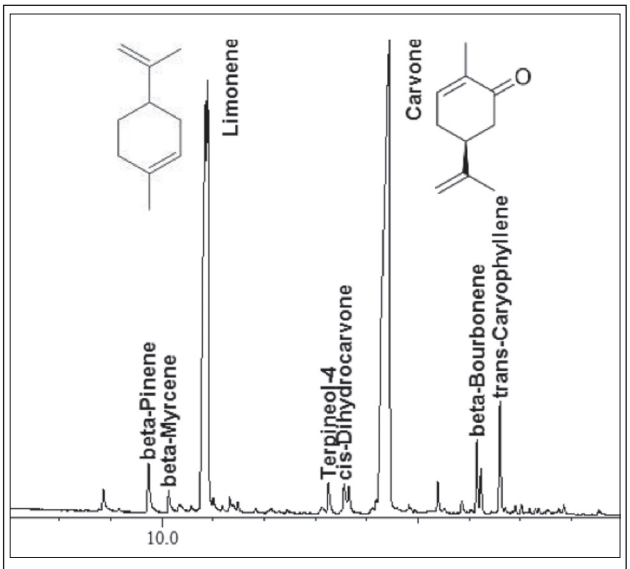


Figure 1 *Chromatogram of SEO analysed with GC-MS.*

Table I *Chemical composition of Algerian Mentha spicata (L.) essential oil using GC-MS.*

RT (min)	Name	%
8.855	α-Pinene	0.66
9.742	β-Pinene	1.97
10.132	β-Myrcene	0.77
10.853	Limonene	24.99
10.899	1.8-Cineole	7.22
11.178	β-Ocimene	0.10
11.324	γ-Terpinene	0.35
11.487	*cis*-Terpineol	0.16
13.253	Terpineol-4	1.22
13.562	*cis*-Dihydrocarvone	1.06
13.655	Dihydrocarveol	0.88
14.187	Pulegone	0.38
14.442	Carvone	52.60
15.392	Dihydrocarvyl acetate	0.82
15.858	*cis*-Carvyl acetate	0.36
16.154	β-Bourbonene	1.87
16.234	β-Elemene	0.96
16.610	*trans*-Caryophyllene	3.38
17.022	α-Humulene	0.25
	Oxygenated Monoterpenes	**64.7**
	Monoterene Hydrocarbons	**28.84**
	Sesquiterpene Hydrocarbons	**6.46**

Tamilnadu (India) and reported carvone (48.60) and limonene (11.30%) as the dominant compounds. Padalia et al. [15] reported the chemical composition of 16 cultivars of Chinese *Mentha* species and revealed that carvone (51.3%–65.1%) and limonene (15.1%–25.2%) are the principal compounds in five cultivars of spearmint. In contradiction with our data, Telci et al. [16] studied chemical composition and antimicrobial effect of SEO and demonstrated that piperitone and pulegone were the main components. Else, El-Sayed et al. [17] confirmed that menthone (32.43%) and eucalyptol (18.79%) were the dominant constituents of the Egyptian SEO. Further, a linalool-rich chemotype (82.8%) was described from Turkey [18].

Usually, the observed changes in chemical composition of SEO, when compared with earlier reports, could be linked to different abiotic or biotic factors comprising several extraction methods, climate and seasonal variations, geographical conditions, relative humidity, agronomic conditions (harvesting time, crop density, organic fertilizers), genotype, stage of the plant growth and processing of plant materials before distillation of the SEO [19].

3.2. In vivo anti-inflammatory activity

3.2.1. In vivo anti-inflammatory activity using carrageenan-induced paw edema test

The anti-inflammatory effect of medicinal herbs has been confirmed and few others refuted. Therefore the present research is carried out with a purpose to screen the effectiveness of anti-inflammatory potential of SEO in experimental animals. The acute inflammation was experimentally made by carrageenan to evaluate the anti-inflammatory property of SEO in mice. Subcutaneous injection of carrageenan produced an increase in animal paw weight due to edema, thus demonstrating acute inflammation.

As shown in *Table II*, SEO showed an interesting anti-inflammatory potential. At 4 hours after oral administration of SEO, the weight of treated left hind paw was similar for 200 µL/kg and 20 µL/kg (3.7±6.3 mg and 02.5±3.48 mg, respectively) with edema inhibition values of 65.87% and 77.24%. This level of edema inhibition was similar to the level detected using 50 mg/kg of the standard reference drug (85.32%). Our findings revealed that treatment with SEO was effective in decreasing the edema induced by carrageenan. However, the drug used as a reference (sodium diclofenac) was more effective in preventing these effects.

The carrageenan-induced paw edema assay is the most commonly used test to determine new anti-inflammatory molecules or drugs. This model is greatly reproducible and has been well recognized as an effective technique to investigate pro-inflammatory cytokine generation and mediators (serotonin, histamine, prostaglandins and bradykinin) in the paw tissue in inflammatory situations [12,20].

Our investigation presents a primary research and additional studies are needed to explain the effect and mechanism of action of the SEO components. Several studies reported that the anti-inflammatory activities obtained with SEO might be related to some anti-inflammatory molecules such as oxygenated monoterpenes (carvone and eucalyptol) [20,21]. In fact, D-carvone inhibited the animal hind paw edema induced by various phlogistics (histamine, carrageenin, dextran and bradykinin) in a dose-dependent manner [20].

In addition, it has been reported that mint oil also prevents the inflammatory consequences of Lipo-polysaccharide (LPS), together with inhibition of prostaglandin E2 (PGE2), interleukin-1 (IL-1) and leukotriene B4 (LTB4) production by LPS-stimulated human monocytes [22,23].

3.2.2. Histological examination of the mice paws after injection of carrageenan

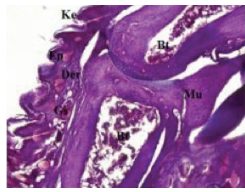
To confirm the anti-inflammatory effect of SEO histologically, paw biopsies were harvested 4 h after carrageenan had been injected. Paw tissues from all the groups of animal were examined by H&E staining. The control groups not induced by inflammation showed normal tissue (*Figure 2A*) with no inflammation, tissue destruction or swelling phenomenon in the paws of normal mice. On the other hand, the animal injected with carrageenan (*Figure 2C*) showed a strong edema and inflamed cavities in the paw tissue, characterized by congestion of blood vessels, dermal and epithelial tissue with an important number of infiltrated inflammatory cells detected in the paw tissue. The cell types were mostly neutrophils, which describe acute inflammation.

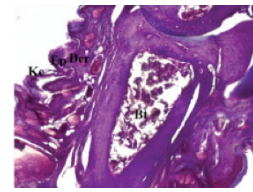
As for the positive (*Figure 2B*) and SEO groups (*Figure 2D*, *E* and *F*), edematous condition was clearly decreased by treatment with NSAID (diclofenac sodium, 50 mg/kg) and SEO (2, 20, and

Table II *In vivo Effects of the spearmint EO (2, 20 and 20 µL/kg) and diclofenac sodium (50 mg/kg) on carrageenan-induced paw edema in mice.*

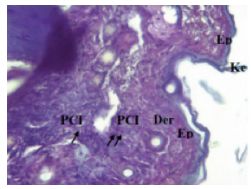
Treatment	Weight (mean, mg) ± SD			
	Left paw	Right paw	Edema weight #	% Inhibition of edema
Negative control (Vehicle)	140.2±18.72	129.3±11.68	10.9±15.19 ^{B}	/
Positive control (NSAID)	132.4±8.99	130.8±10.00	1.6±3.21 ^{A}	85.32
SEO 200	134.1±13.38	130.4±17.54	3.7±6.30 ^{AB}	65.87
SEO 20	139.7±12.39	137.2±15.07	2.5±3.48 ^{AB}	77.24
SEO 2	140.1±12.08	132.9±18.34	7.2±14.03 ^{AB}	33.57

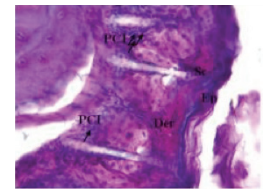
Groups of mice (n = 6/group) were pretreated with vehicle (NaCl, 0.9%), NSAID: non-steroidal anti-inflammatory drugs (Sodium diclofenac, 50 mg/kg, *p.o.*). SEO: Spearmint essential oil at doses of 2, 20, and 200 µL/kg (*p.o.*) # Means within the same column followed by the same capital letter are not significantly different ($p>0.05$) according to ANOVA one way analysis followed by Tukey's *post hoc* multiple comparison test.





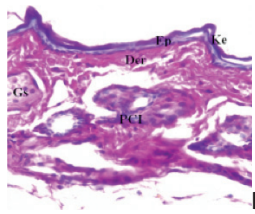
Right paw (control) (Gx10) = Edema (-); inflammatory cell infiltration (-), inflammation phase (-).

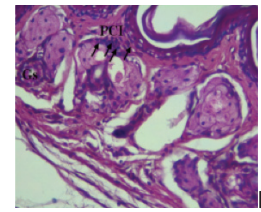




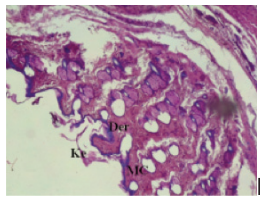
Positive control treatment (sodium diclofenac) (Gx40) Edema (±); inflammatory cell infiltration (+), inflammation phase (±).

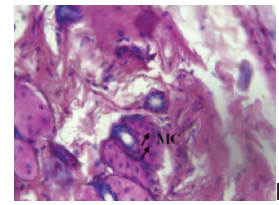
Negative control = λ-carrageenan group (Gx40) Edema (++); inflammation phase (+++); inflammatory cell infiltration (+++) in epidermal and dermal layers, muscle and cartilage.



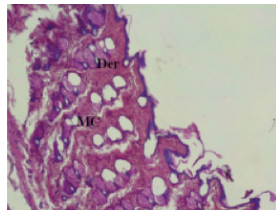


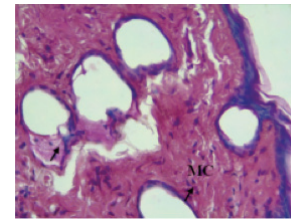
SEO treatment (2 µL/Kg) (D1 x10; D2 x10) Edema (±); inflammatory cell infiltration (+), inflammation phase (±).





SEO treatment (20 µL/Kg) (E1 x10; E2 x40) Edema (±); inflammatory cell infiltration (+), inflammation phase (±).





SEO treatment (200 µL/Kg) (F1 x10; F2 x40) Edema (±); inflammatory cell infiltration (+), inflammation phase (±).

Ke: keratin; Ep = epidermal layer; SC = subcutaneous layer; Bt = bone tissue; Mu = muscle fibers; Gs = sebaceous gland; PCI = polymorphonuclear cells infiltration; MC = mononuclear cells; Ed: edema.

Figure 2 *Histopathological examinations on λ-carrageenan-induced paw tissue swelling, edema and neutrophil infiltration. Light photomicrographs of the H&E stained paw tissue in the (A) control group (right paw), (B) NSAIDs group, (C) λ-carrageenan group, (D) SEO (2 µL/kg), (E) SEO (20 µL/kg) and (F) SEO (200 µL/kg) groups. Paws were harvested 4 h after injection of carrageenan and subjected to histochemical staining.*

200 µL/kg) as well as a decrease in the infiltration of inflammatory cells. At higher dose of SEO, treated animals showed a better effect in decreasing of neutrophils as compared with standard drug (*Figure 2F* vs *2C*).

Our findings clearly establish the property of SEO within the target tissue, giving additional confirmation that SEO reduces carrageenan-induced paw edema. Mogosan et al. [12] stated that the treatment of SEO inhibited the edema and decreases the cellular infiltrates probably by decreasing the inflammatory chemical mediators.

The histopathological findings also substantiate with the paw edema analyses. Histopathological examinations indicated that edema formation and, inflammatory cell infiltration were obviously suppressed in the animal treated with the SEO. These data support the results obtained from the paw edema test and confirm the anti-inflammatory action of SEO against acute inflammation.

The use of current NSAIDs is frequently associated with severe and different side effects. Hence alternative therapeutic molecules are required. Few studies have been initiated around the world into researching, evaluating and analyzing the local medicinal plants with anti-edematous values. In spite of the medicinal potential of SEO, its effect on inflammation has not been previously reported in detail. Therefore, in the present study, we screened and confirmed for the first time the anti-inflammatory efficacy of SEO against carrageenan induced paw edema followed by histological analysis.

3.2.3. In vivo anti-inflammatory activity using xylene-induced ear edema assay

The anti-inflammatory effect of SEO was further assessed by the inhibition of xylene-induced ear edema. As shown in *Table III*, topical application of xylene in the control group caused

Table III *Effects of the SEO (2, 20 and 200 µL/kg) and Ketoprofen (5 mg/ear) on xylene-induced ear edema in mice.*

Treatment	Weight (mean, mg) ± SD			
	Right ear	Left ear	Edema weight #	% inhibition of edema
Negative control (Vehicle)	9±0.44	12.6±1.34	3.6±1.14 ^{B}	-
Positive control (NSAID)	7.4±1.51	7.8±1.09	0.4±1.14 ^{A}	**88.88**
SEO 200 µL/kg	8±0.83	8.2±0.83	0.2±0.54 ^{A}	**94.44**
SEO 20 µL/kg	8±0.54	8.4±0.54	0.4±0.44 ^{A}	**88.89**
SEO 2 µL/kg	7.4±0.89	8.8±0.83	1.4±1.14 ^{A}	**61.11**

Groups of mice (n = 6/group) were pretreated with vehicle, NSAID: non-steroidal anti-inflammatory drug (Ketoprofen 2.5%). SEO: Spearmint essential oil at doses of 2, 20, and 200 µL/kg (topically). # Means within the same column followed by the same capital letter are not significantly different (p>0.05) according to ANOVA one way analysis followed by Tukey's *post hoc* multiple comparison test.

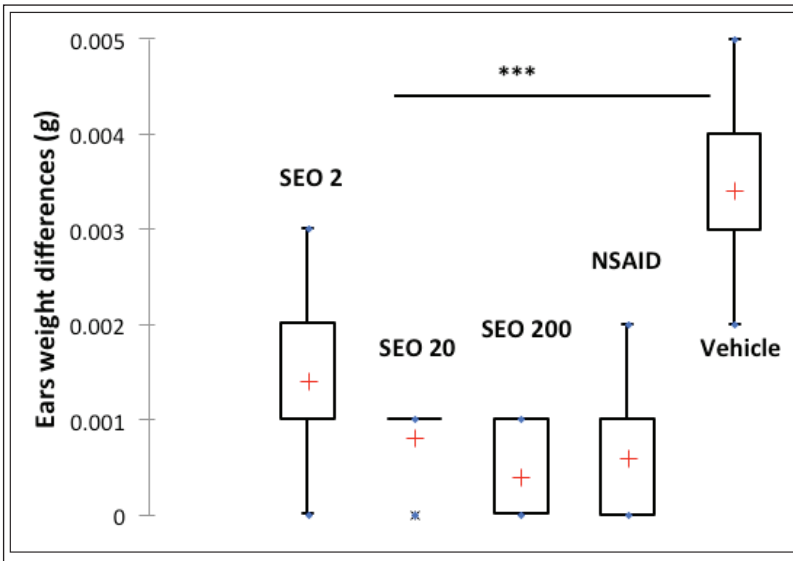


Figure 3 *Topical anti-inflammatory effect of SEO in xylene-induced ear edema. Vehicle: control group (mice treated with almond oil topical application); SEO: mice treated with spearmint essential oil topically at different doses (2, 20, and 200 µL/kg) per ear; NSAID: mice treated with non-steroidal anti-inflammatory drugs (ketoprofen 2.5%). Values represent the mean ± SEM. *** significant difference (p<0.001) according to ANOVA one-way analysis followed by Tukey's post hoc multiple comparison tests.*

a marked increase in the weight of the ears. However, the topical administration of the SEO (200, 20 and 2 µL/kg), 40 minutes before xylene application, decreased the development of ear edema. The inhibitory effect of the oil was similar to the inhibition caused by ketoprofen (*Figure 3*). Treatment of the mice with a high dose of SEO suppressed xylene-induced ear edema, reducing swelling by 94.44% (p<0.001). The data indicate that SEO possesses inhibitory effects against acute inflammation.

Our findings revealed that SEO can markedly prevent the formation of xylene-induced ear edema. Mice ear edema may comprise the release of pro-inflammatory chemical mediators, increasing vascular permeability, leukocytes infiltration, plasma leakage and the production of cytokines such as interleukin 1β, tumor necrosis factor alpha (TNF α) [24]. SEO may produce the anti-inflammatory action by influencing the actions of the above mediators [22,25].

NSAIDs are usually among the most used drugs in the treatment of inflammation. Unfortunately, the use of NSAIDs is restricted by several adverse effects and about 20% of patients will develop gastric ulcer [12]. Our study demonstrated that SEO showed similar effects with NSAIDs on the two models of inflammation (carrageenan and xylene).

Table IV *Study of anti-nociceptive activity of SEO by acetic acid-induced writhing test*

Treatment	Number of writhing (Mean ± SD) #	Inhibition (%)
Negative control (acetic acid)	77.8 ± 5.40 ^{B}	-
Positive control (Phloroglucinol)	55.6 ± 9.09 ^{A}	28.53 ***
SEO 200 µL/kg	59.4 ± 5.89 ^{A}	23.65 **
SEO 20 µL/kg	65.2 ± 3.96 ^{A}	16.19 *
SEO 2 µL/kg	65.24 ± 3.96 ^{AB}	16.14 ^{ns}

SEO: mice treated with spearmint essential oil at different doses (2, 20, and 200 µL/kg, *p.o*). Values are expressed as mean ± SD (n = 6, per group); * p<0.05; ** p<0.01; *** p<0.001. # Means within the same column followed by the same capital letter are not significantly different (p>0.05) according to ANOVA one way analysis followed by Tukey's *post hoc* multiple comparison test.

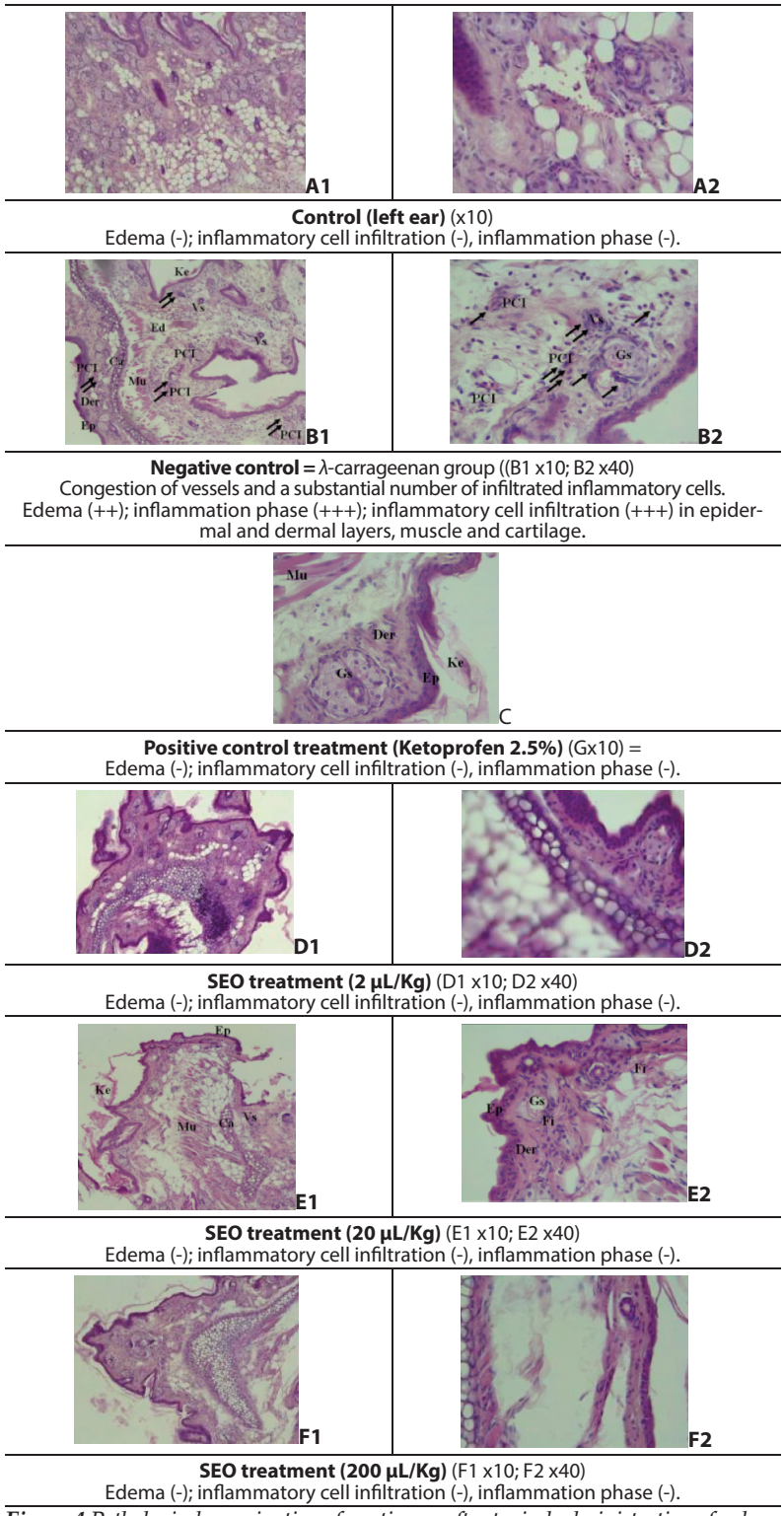


Figure 4 *Pathological examination of ear tissues after topical administration of xylene.*

Histopathology sections of mice ear biopsies showing keratin, epidermal, dermal, muscle, and cartilage layers. Hematoxylin & Eosin stained sections were scored as mild (+), modest (++), and severe (+++) for edema and substantial inflammatory polymorphonuclear cell infiltration (PCI) in the dermis inflammation phase.

(A) Normal Ear; (B) Control: Topical Application of xylene (20 µL/ear) showed induced inflammatory lesion with edema and infiltration of polymorphonuclear leukocytes; (C) Ketoprofen decreased the indicated changes; Topical Application of the SEO at doses of 2 (D), 20 (E) and (F) 200 µL/kg after 4 h of topical xylene was able to decrease epidermis thickness, edema, and infiltration of polymorphonuclear leukocytes. Ke: keratin; Ep: epidermal layer; Bo: bone tissue; PCI: polymorphonuclear cells infiltration; Ed: edema; Mu: muscle.

3.2.4. Examining the mouse ear tissue histology

Anti-inflammatory effect of SEO on the carrageenan test led us to determine this effect in another neurogenic inflammatory assay (xylene-induced ear edema). We investigated H&E stained ear sections from xylene-induced ear edema (*Figure 4*). By histological comparison, topical application of SEO decreased paw thickness and associated pathological indicators (*Figure 4D*, *E* and *F*) comparable to the positive control (ketoprofen gel) (*Figure 4C*).

The ear edema model induced by xylene has certain advantages in the determination of NSAIDs and has better predictive values in the screening of anti-inflammatory new molecules [23]. Xylene-induced swelling is followed by innate immunity reaction of the skin, a cytotoxicity response of activated T cells followed by the migration of polymorphonuclear leucocytes which enhance inflammation and swelling of the ear. The pathological examination of inflamed tissues (paws and ears) showed that SEO inhibited infiltration of polymorphonuclear leucocytes into the site of inflammation. Therefore, another option is that SEO exerts its anti-edematous action partly through the inhibition of polymorphonuclear cell infiltration [22,23,25]. In addition, the chemical composition of SEO revealed the presence of high quantity of oxygenated terpene compounds such as carvone, menthol and eucalyptol. Literature review showed that the presence of these compounds in the SEO may be the main reason of its important anti-inflammatory property [21].

3.3. In vivo effect of SEO on acetic acid-induced writhing response

Acetic acid induced writhing experiment was done to evaluate the peripheral analgesic property of SEO. It is well known that acetic acid in some

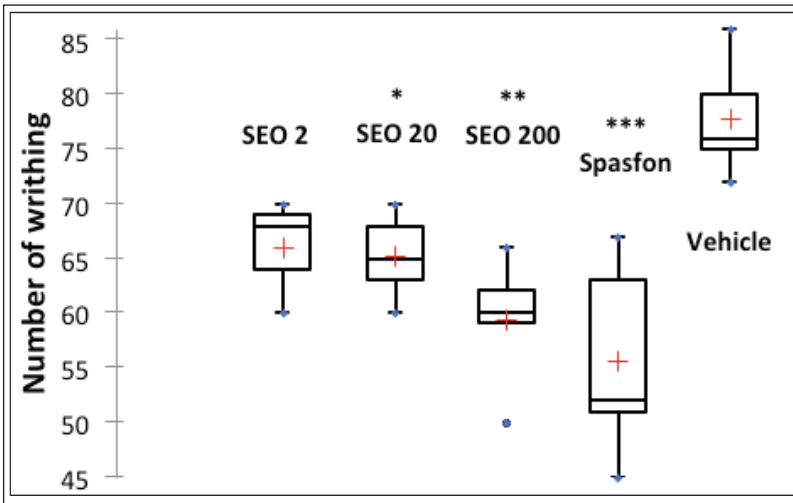


Figure 5 *Anti-nociceptive activity of SEO in the acetic acid induced writhing test in vivo.*

*The effect of the SEO (2, 20 and 200 µL/kg, i.p.), and Phloroglucinol (Spasfon® 80 mg/kg, i.p.) in the acetic-acid-writhing-induced nociception test in mice. Values are expressed as mean ± SD (n = 6, per group); * p<0.05; ** p<0.01; *** p<0.001 significantly different from the control group, according to ANOVA, followed by Tukey's test.*

way is responsible for secretion of endogenous mediators thereby stimulating the neurons responsible for pain perception, which are receptive to NSAIDs [26]. In this research, SEO showed a significant *in vivo* analgesic effect using the acetic acid-induced writhing test. Intraperitoneal (i.p) injection of acetic acid produced 77.8±5.40 writhes in the solvent control group (*Table IV* and *Figure 5*). The writhing response was significantly decreased by pretreatment with 200 µL/kg of SEO (77.8±5.40; $p<0.01$). Standard group which received Spasfon (Phloroglucinol) showed 28.53% ($p<0.001$) of inhibition in writhing movement.

The analgesic properties of mint species have been studied and demonstrated by several authors [12,27]. In this study, we evaluated the anti-nociceptive effect of SEO using the acetic-acid induced pain model. The oral administration of SEO produced significant inhibition of the acetic acid-induced abdominal writhing in dose dependent manner in the mice, but the inhibition was similar to that produced by Phloroglucinol only at a higher dose (200 µL/kg). These data suggest that SEO may produce peripheral analgesic activity by inhibiting the chemical mediators and/or cytokines (histamine, serotonin, bradykinin, cytokines, and eicosanoids). These mediators stimulate an increase of vascular permeability as well as reduce the threshold of nociception and stimulate the nervous terminal of nociceptive fibers [7,23]. The anti-inflammatory and anti-nociceptive activities of SEO may be related to the synergy interactions of its major or minor chemical components such as carvone, limonene and eucalyptol [28].

4. Conclusion

Our findings clearly confirmed that SEO has a potent peripheral analgesic activity and also demonstrated systemic and local anti-inflammatory properties in carrageenan and xylene models and these preliminary data could provide some credit for the potential use of spearmint as a bioactive agent for the treatment-prevention of inflammatory and painful conditions. Additional cellular and molecular assays will be done to investigate its real mechanisms of SEO including its active components such as carvone and limonene, alone or in combination, and to characterize the receptors involved in the anti-nociceptive and anti-inflammatory effects. A systemic study is required to produce nutraceuticals drug from SEO for treating various health problem of human kind.

Sources of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not for-profit sectors.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

The experimental assay was agreed and approved by the European guidelines for animal handling and experiments.

Acknowledgement

The authors are grateful to SAIDAL pharmaceutical company (Research & Development Center, Algiers, Algeria) for the excellent research facilities. The authors are grateful to the Hospital of Kolea (Tipaza, Algeria) for providing technical assistance in pathological analysis.

Authors' contributions

Study concept and design: SK, MNB and MAF; Experiments: SK, AB and MNB; Analysis and interpretation of data: SK, AB and FB; Drafting of the manuscript: MNB, FB, and WS; and Critical revision of the manuscript for important intellectual content: SK and WS. All authors critically revised the article for important intellectual content and approved the final version.

REFERENCES

1. Jan, S., & Khan, M. R. Antipyretic, analgesic and anti-inflammatory effects of Kickxia ramosissima. J Ethnopharmacol. 2016; 182: 90-100. https://doi.org/10.1016/j.jep.2016.02.020
2. Hotamisligil, G. S. Inflammation, metaflammation and immunometabolic disorders. Nature. 2017; 542: 177. https://doi.org/10.1038/nature21363
3. Laskin, D. L., & Pendino, K. J. Macrophages and inflammatory mediators in tissue injury. Ann Rev Pharmacol Toxicol. 1995; 35: 655-677. https://doi.org/10.1146/annurev.pa.35.040195.003255
4. Shrihari, T. G. Dual role of inflammatory mediators in cancer. Ecancermedicalscience. 2017; 11: 721. https://doi.org/10.3332/ecancer.2017.721
5. Wongrakpanich, S., Wongrakpanich, A., Melhado, K., & Rangaswami, J. A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. Aging Dis. 2018; 9: 143. http://dx.doi.org/10.14336/AD.2017.0306
6. Bruno, A., Tacconelli, S., & Patrignani, P. Variability in the response to non-steroidal anti-inflammatory drugs: mechanisms and perspectives. Basic Clin Pharmacol Toxicol. 2014 ; 114: 56-63. https://doi.org/10.1111/bcpt.12117
7. Mahboubi, M. *Mentha spicata* as natural analgesia for treatment of pain in osteoarthritis patients. Compl. Therap. Clin Prac. 2017; 26: 1-4. https://doi.org/10.1016/j.ctcp.2016.11.001
8. Brahmi, F., Adjaoud, A., Marongiu, B., Falconieri, D., Yalaoui-Guellal, D., Madani, K., & Chibane, M. Chemical and biological profiles of essential oils from *Mentha spicata* L. leaf from Bejaia in Algeria. J Essent Oil Res. 2016; 28: 211-220. https://doi.org/10.1080/10412905.2015.1118411
9. Scherer, R., Lemos, M. F., Lemos, M. F., Martinelli, G. C., Martins, J. D. L., & da Silva, A. G. Antioxidant and antibacterial activities and composition of Brazilian spearmint (*Mentha spicata* L.). Ind Crops Prod. 2013 ; 50: 408-413. https://doi.org/10.1016/j.indcrop.2013.07.007
10. Nikšić, H., Durić, K., Omeragić, E., Nikšić, H., Muratović, S., & Bečić, F. Chemical characterization, antimicrobial and antioxidant properties of *Mentha spicata* L.(Lamiaceae) essential oil. Bull Chem Technol Bosn Herzeg. 2018; 43-48.
11. Bardaweel, S. K., Bakchiche, B., ALSalamat, H. A., Rezzoug, M., Gherib, A., & Flamini, G. Chemical composition, antioxidant, antimicrobial and antiproliferative activities of essential oil of *Mentha spicata* L.(Lamiaceae) from Algerian Saharan atlas. BMC Compl Altern Med. 2018; 18: 201. https://doi.org/10.1186/s12906-018-2274-x
12. Mogosan, C., Vostinaru, O., Oprean, R., Heghes, C., Filip, L., Balica, G., & Moldovan, R. I. A comparative analysis of the chemical composition, anti-inflammatory, and antinociceptive effects of the essential oils from three species of *Mentha* cultivated in Romania. Molecules. 2017; 22: 263. https://doi.org/10.3390/molecules22020263
13. Boukhatem, M. N., Ferhat, M. A., Kameli, A., Saidi, F., & Kebir, H. T. Lemon grass (Cymbopogon citratus) essential oil as a potent anti-inflammatory and antifungal drugs. Libyan J Med. 2014; 9: 25431. https://doi.org/10.3402/ljm.v9.25431
14. Govindarajan, M., Sivakumar, R., Rajeswari, M., & Yogalakshmi, K. Chemical composition and larvicidal activity of essential oil from *Mentha spicata* (Linn.) against three mosquito species. Parasitol Res. 2012; 110: 2023-2032. https://doi.org/10.1007/s00436-011-2731-7
15. Padalia, R. C., Verma, R. S., Amit, C., Velusamy, S., & Chanotiya, C. S. Essential oil composition of sixteen elite cultivars of *Mentha* from western Himalayan region, India. Maejo Int J Sci Technol. 2013; 7: 83-93. https://doi.org/10.14456/mijst.2013.7
16. Telci, I., Demirtas, I., Bayram, E., Arabaci, O., & Kacar, O. Environmental variation on aroma components of pulegone/piperitone rich spearmint (*Mentha spicata* L.). Ind Crops Prod. 2010; 32: 588-592. https://doi.org/10.1016/j.indcrop.2010.07.009
17. El-Sayed, Z. I. A., Omar, N. A., & Romeh, A. A. Chemical constituents and biocidal activity of the essential oil of *Mentha spicata* L. grown in Zagazig region, Egypt. Res J Agr Biol Sci. 2009; 5; 1089-1097.
18. Baser, K. H. C., Kürkçüoglu, M., Tarimcilar, G., & Kaynak, G. Essential oils of *Mentha* species from Northern Turkey. J Essent Oil Res. 1999; 11:, 579-588. https://doi.org/10.1080/10412905.1999.9701218
19. Yahia, I. B. H., Jaouadi, R., Trimech, R., Boussaid, M., & Zaouali, Y. Variation of chemical composition and antioxidant activity of essential oils of *Mentha* x *rotundifolia* (L.) Huds.(Lamiaceae) collected from different bioclimatic areas of Tunisia. Biochem Syst Ecol. 2019; 84: 8-16. https://doi.org/10.1016/j.bse.2019.03.001
20. Zhao, M., & Du, J. Anti-inflammatory and protective effects of D-carvone on lipopolysaccharide (LPS)-induced acute lung injury in mice. J King Saud Univ-Sci. 2019; https://doi.org/10.1016/j.jksus.2019.12.016
21. Andrade, L. N., & De Sousa, D. P. A review on anti-inflammatory activity of monoterpenes. Molecules. 2013; 18: 1227-1254. https://doi.org/10.3390/molecules18011227
22. Arumugam, P., Priya, N. G., Subathra, M., & Ramesh, A. Anti-inflammatory activity of four solvent fractions of ethanol extract of *Mentha spicata* L. investigated on acute and chronic inflammation induced rats. Envir Toxicol Pharmacol. 2008; 26: 92-95. https://doi.org/10.1016/j.etap.2008.02.008

23. Yousuf, P. M. H., Noba, N. Y., Shohel, M., Bhattacherjee, R., & Das, B. K. Analgesic, anti-inflammatory and antipyretic effect of *Mentha spicata* (Spearmint). J Pharm Res Int. 2013; 854-864. https://doi.org/10.9734/BJPR/2013/4640

24. Saito, A., Tanaka, H., Usuda, H., Shibata, T., Higashi, S., Yamashita, H., & Nagai, H. Characterization of skin inflammation induced by repeated exposure of toluene, xylene, and formaldehyde in mice. Envir Toxicol. 2011; 26: 224-232. https://doi.org/10.1002/tox.20547

25. Pearson, W., Fletcher, R. S., Kott, L. S., & Hurtig, M. B. Protection against LPS-induced cartilage inflammation and degradation provided by a biological extract of *Mentha spicata*. BMC Complem Altern Med. 2010; 10: 19. https://doi.org/10.1186/1472-6882-10-19

26. Lenardão, E. J., Savegnago, L., Jacob, R. G., Victoria, F. N., & Martinez, D. M. Antinociceptive effect of essential oils and their constituents: an update review. J Braz Chem Soc. 2016; 27: 435-474. https://doi.org/10.5935/0103-5053.20150332

27. Sousa, P. J. D. C., Linard, C. F. B. M., Azevedo-Batista, D., Oliveira, A. C., Coelho-de-Souza, A. N., & Leal-Cardoso, J. H. Antinociceptive effects of the essential oil of Mentha x villosa leaf and its major constituent piperitenone oxide in mice. Braz J Med Biol Res. 2009; 42: 655-659. https://doi.org/10.1590/S0100-879X2009000700010

28. de Sousa, D. P., Mesquita, R. F., de Araújo Ribeiro, L. A., & de Lima, J. T. Spasmolytic activity of carvone and limonene enantiomers. Nat Prod Comm. 2015 ; 10. https://doi.org/10.1177/1934578X1501001120

Stability study of nasal powder formulation containing nanosized lamotrigine

PÉTER GIESZINGER, GÁBOR KATONA, PIROSKA SZABÓ-RÉVÉSZ, RITA AMBRUS*

Institute of Pharmaceutical Technology and Regulatory Affairs, University of Szeged, Faculty of Pharmacy, Szeged, Hungary

**Corresponding author: Rita Ambrus*
E-mail: arita@pharm.u-szeged.hu

Received: *7 April 2020 / Revised: 27 May 2020 / Accepted: 27 May 2020*

Abstract
Drug administration through the nose offers great possibilities which have been discovered in the past few decades. Besides the most known local effect, systemic and central nervous system effect is also available, the administration is non-painful and the degradation effect of the gastrointestinal tract can be avoided. Amongst the nasal formulations, powders have become more popular as their stability is favorable compared to the liquid formulations and a higher doses can be administered in powder form. The quality insurance and stability of the products in the pharmaceutical field have gained considerable attention in the last decades. Due to this fact, the aim was to execute a long-term stability study of a previously developed, nanosized lamotrigine (LAM) containing nasal powder (NP) formulation. The results of the stability test showed that the NP formulation preserved its key properties (particle size, morphology, structure and in vitro drug release) after 6 months of storage.

Keywords: *nasal delivery, stability test, nanotechnology, nasal powder, lamotrigine*

1. Introduction

The nose offers a great possibility to avoid adverse events and increase patient compliance[1–4]. Due to its advantageous properties local, systemic and central nervous (CNS) system effects are also available [5–7]. The application of innovative and efficient products, that are containing nanoparticles, may lead to the improvement of different therapies [8,9]. The quality insurance of pharmaceutical products has received considerable attention in the past few years. That is why the stability of the formulations has become extremely important and therefore, quality influencing parameters need to be kept constant during the transport, storage, and application. Generally, solid dosage forms (e.g. nasal powders) have better stability than liquid formulations [10–12].

In our previous studies, a nanosized lamotrigine (LAM) containing nasal powder (NP) product was researched and developed. The investigations of the product showed that due to the effect of milling, the nanosized LAM particles became partly amorphous and distributed homogenously on the polymer, polyvinyl alcohol (PVA) matrix. Moreover, the product performed rapid and high amount of drug release *in vitro* and *in vivo*. This study aimed to carry out the long-term stability study of the previously developed NP formulation, which contained nanosized LAM [13–15].

2. Materials and methods

2.1. Materials

Lamotrigine, poorly water-soluble (0.17 mg/mL at 25 °C) was purchased from Teva Ltd. (Budapest, Hungary). PVA (Mw = 27,000), water-soluble synthetic polymer – that was applied to stabilize the unique drug particles, thus improving their absorption – was supplied by ISP Customer Service GmBH (Cologne, Germany).

2.2. Sample Preparation

PVA was used as an additive during the sample preparation process to maintain the stability and individuality of LAM particles. NP sample was produced as follows: 0.8 g PVA and 1 g LAM were mixed in a Turbula mixer (Turbula System Schatz; Willy A. Bachofen AG Maschinenfabrik, Basel, Switzerland) using 60 rpm for 10 min. After mixing, the sample was placed into a planetary ball mill (Retsch PM 100; Retsch, Neuhausen, Germa-

DOI: 10.33892/aph.2020.90.27-31

ny) and milled in a 50 mL capacity milling chamber for 1.5 h on 400 rpm with 10 steel balls (diameter 10 mm, the weight of each ball 4.02 g). In the case of the physical mixture (PM), PVA was milled for 1.5 h on 400 rpm and then—according to our previous experiments—it was mixed with unmilled LAM using the same Turbula mixer for 10 min on 60 rpm.

2.3. Circumstances of the stability

Stability tests were performed according to the ICH Q1A guideline [16] in Binder KBF 240 (Binder GmbH, Tuttlingen, Germany) equipment, with a constant-climate chamber. An electronically controlled APT.line™ line preheating chamber and refrigerating system ensured temperature accuracy and reproducibility of the results in the temperature range between 10 and 70 °C and the relative humidity (RH) range between 10 and 80%. The stability test was performed at 25 ± 2 °C with 50 ± 5% RH (room conditions). Samples were kept in a closed jar during the tested period. Sampling was carried out after 1 day; 3 and 6 months.

2.4. Particle size and morphology characterization

The particle size of the microparticles was characterized by using Leica Image Processing and Analysis System device (Leica Q500MC; Leica Microsystems, Wetzlar, Germany). The test parameters of 300 particles were their length, width, area, and district/convex perimeter.

The morphology and the size the LAM nanoparticles – that were on the surface of the polymer microparticles – were investigated by SEM (Hitachi S4700; Hitachi Ltd., Tokyo, Japan) at 10 kV. The samples were gold–palladium-coated (90 s) with a sputter coater (Bio-Rad SC502; VG Microtech, Uckfield, UK) using an electric potential of 2.0 kV at 10mA for 10 min. The air pressure was 1.3–13.0 mPa. Distribution of LAM particle diameter was obtained by analyzing SEM images with the ImageJ software (1.50i; Java 1.6.0_20 [32-bit]; Windows NT) environment using approximately 500 particles [17].

Statistical analysis was performed with TIBCO Statistica® 13.4 (Statsoft Hungary, Budapest, Hungary). All reported data are means ± SD. The Student's t-test was used to determine the statistical significance. Changes were considered statistically significant at $p < 0.05$.

2.5. Structural investigations

2.5.1. Thermoanalytical measurements

The Mettler Toledo TGA/DSC 1 thermal analysis system (Mettler-Toledo GmbH, Greifensee, Switzerland) was applied to characterize the structures of the products. The DSC (differential scanning calorimetry) and TG (thermogravimetry) measurements were made by examining approximately 3-5 mg of samples in the temperature range between 25 °C and 230 °C at a heating rate of 5 °C/min under constant argon flow of 150 mL/min and nitrogen flow of 50 mL/min. Data analysis was performed using the STARe software (Mettler-Toledo GmbH, Greifensee, Switzerland). The cristallinity indexes were calculated based on the ratio Normalized integrals, where the PM samples was considered as 100%.

2.5.2. X-ray powder diffraction (XRPD)

The XRPD measurement was carried out with a BRUKER D8 advance X-ray powder diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) with Cu K λI radiation (λ=1.5406 Å) and a VÅNTEC-1 detector (Bruker AXS GmbH, Karlsruhe, Germany). The powder samples were loaded in contact with a plane quartz glass sample slide with an etched square and measured. Samples were scanned at 40 kV and 40 mA. The angular range was 3°–40° 2θ, at a step time of 0.1 seconds and a step size of 0.007°. All manipulations, including Kα2 stripping, background removal and smoothing of the area under the peaks of the diffractograms, were performed using the DIFFRACplus EVA software. The crystallinity index (Xc) values were calculated based on the following formule, where A marks the area under the whole curve:

$$X_c = A_{crystalline} / (A_{crystalline} + A_{amorphous}) * 100$$

PM sample was considered as 100%.

2.6. In vitro release study

The modified paddle method (USP dissolution apparatus, type II; Pharma Test, Hainburg, Germany) was used to examine the dissolution rate of LAM-containing co-milled nasal powders and determine the drug release profile from the samples. The test was carried out under nasal conditions

Table 1 *The results of particle size investigation of the product.*

	Average size of the product (μm)	t-value	p-value	Significance
1-day	29.91±15.85	-0.1435	0.8883	n.s.
3 months	28.48±12.81	0.2994	0.7690	n.s.
6 months	26.52±11.14	0.9064	0.3801	n.s.

In the table n.s means that there is no significant difference at 95% level.

Table 2 *The results of particle size investigation of LAM.*

	Average size of LAM (nm)	t-value	p-value	Significance
1-day	97±60	1.2382	0.2347	n.s.
3 months	105±77	0.7934	0.4408	n.s.
6 months	120± 84	-0.0408	0.9687	n.s.

In the table n.s means that there is no significant difference at 95% level.

for temperature and pH. 100 ml phosphate-buffered saline solution (PBS of pH 5.60 at 30 °C) was used as a medium in 150 mL vessels, in which 108 mg of the samples were tested. The paddle was placed to halfway through the medium and was rotated at 50 rpm, and the sampling points were at 5 min, 10 min, 15 min, 30 min, 45 min, and 60 min. In each sampling point 2 mL samples were taken, which volume was immediately replaced with fresh buffer solution to maintain the permanent volume. Cellulose ester membranes with 0.45 μm pore diameter was used for filtration. The sampling points were more frequent in the beginning of the investigation as the mucociliary clearance renews the mucus every 15 min. The following sampling points offered extra information about the dissolution behavior of LAM. After filtration, the drug content of the aliquots was determined using spectrophotometry (Unicam UV/VIS Spectrophotometer) at 307 nm. The tests were carried out in triplicates.

3. Results and discussion

3.1. Characterization of particle size and morphology

The results of particle size determination resulted in same particle size of the product during the examined period (*Table 1*), which shows that the product's particles did not aggregate. The size falls into the range which is desired in the case of nasal powders, which is 10-40 μm [17].

The particle size of LAM in the formulation showed an increase with relatively high standard deviation, which is related to the nanoscale range and the ImageJ technique used (*Table 2*). However, according to results of the statistical analysis there is no significant difference in the particle sizes during the storage period, thus the previously experienced rapid and high degree of release can be predicted.

The SEM pictures confirm the results of the particle size determination as the nanosized LAM particles can be seen on the PVA surface in a homogenous distribution and showed no sign of aggregation during the tested period (*Figure 1*).

3.2. Structural investigations

The DSC curves (*Figure 2A*) in the tested period shows that the partly amorphous property of the sample did not change considerably. The melting point of LAM was between 216 °C and 217 °C, which became lower (~ 204 °C) due to the effect of milling and the presence of PVA. The two melting points merged as LAM melted on ~204 °C, while PVA did it on ~210 °C. However, the character and area of the endothermic peak are almost the same in each sampling point in the case of the NP formulation. Compared to the PM, which was regarded as 100%, the crystallinity degrees of the samples were between 42 and 48%.

This immutability can be seen in the case of XRPD diffractograms (*Figure 2B*). According to the

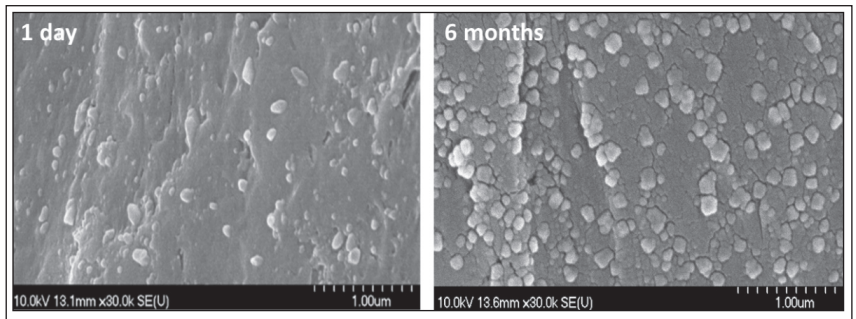


Figure 1 *The SEM pictures of the NP samples*

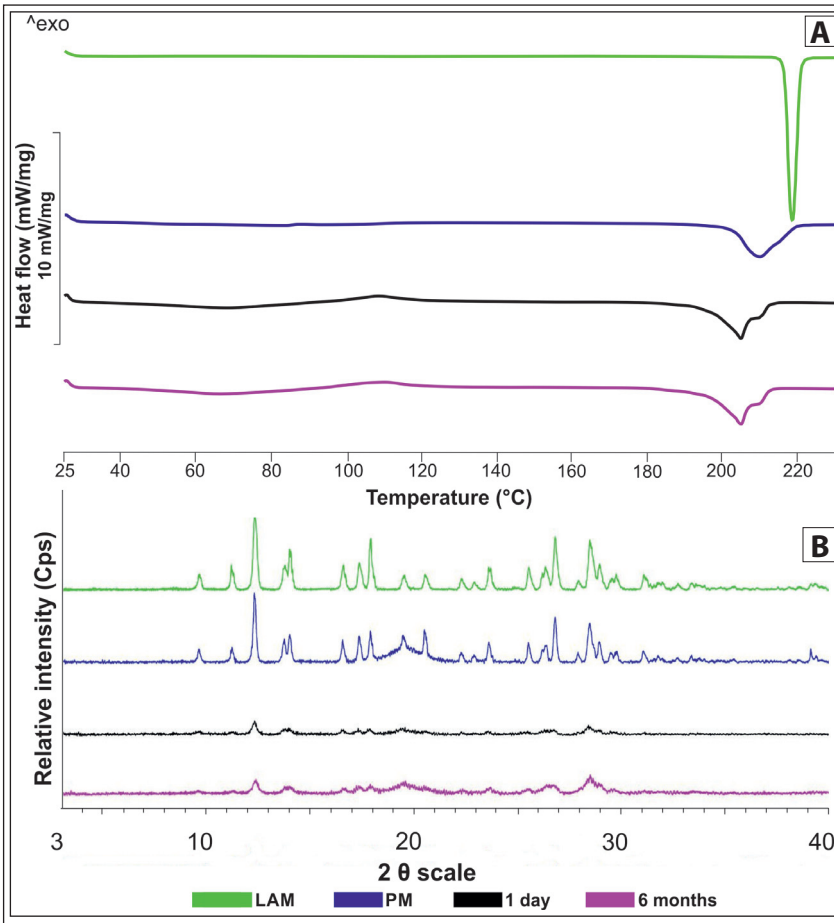


Figure 2 *The DSC curves (A) and the XRPD diffractograms (B) of the samples*

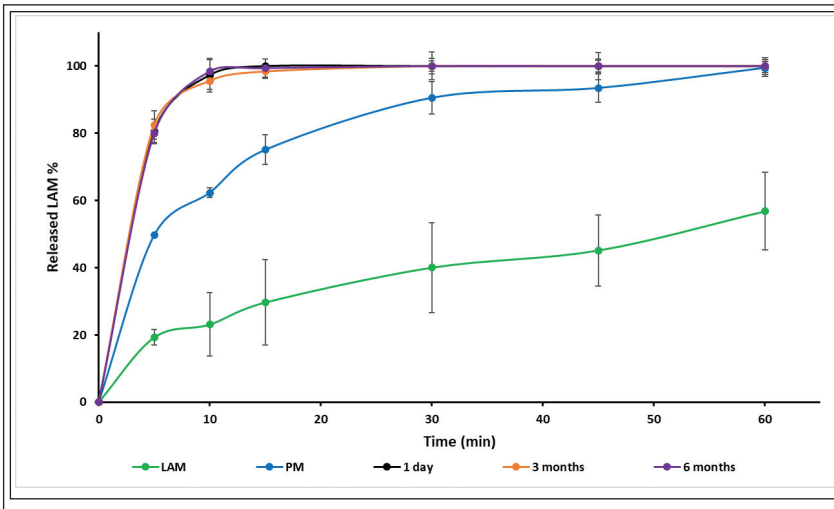


Figure 3 *The results of the release study, where LAM is the raw lamotrigine and PM is the Physical Mixture*

Table 3 *The crystallinity index (X_c) values of the samples*

	X_c (%)	
	DSC	**XRPD**
PM	100	100
1-day	41.91	47.58
6 months	47.53	48.40

diffractograms, the characteristic 2θ° values of the raw LAM were at 12.57, 19.62, 27.04, 27.19 and 28.92 [13], which can be also seen in the case of the PM. Due to the co-milling with PVA, the crystallinity of the LAM decreased as the intensity of the characteristic peaks decreased in the 1-day sample. After 6 months, this decreased crystallinity was maintained. The crystallinity index was found 47.58% in the 1-day sample, which value did not change considerably during the storage.

Table 3 shows the crystallinity index values of the samples. The results of the measurement confirmed the DSC results, which means that the LAM became and remained partly amorphous due to the milling effect and the presence of PVA in the tested period. Moreover, the TG results showed no change in mass, which refers that no dehydration or decomposition occurred during the investigation.

3.3. In vitro release study

In *Figure 3*, it can be seen that the LAM from the surface of the polymer released rapidly in a high amount in the case of the NP sample compared to the PM and raw LAM. This rapid release was maintained during the examined period as practically there is no difference between the samples. The rapid dissolution rate was occurred due to the nanosized property of LAM and the presence of PVA.

4. Conclusion

To conclude the study, it can be said that the key properties of the NP product did not change considerably. The particle size of the product did not show relevant change during the tested period, while the particle size of LAM in the formulation

showed an increase with no significant difference. Moreover, the partly amorphous property of LAM was maintained during the stability test and according to the TG results, no dehydration or decomposition occurred, which suggests that the sample did not remove water from its surroundings during storage. The results of the dissolution studies showed a rapid and high amount of the released drug in the examined period. The results predict that PVA could prevent the particles from aggregation and recrystallization. However, further stability investigations are required – according to ICH guidelines –, but it they will be worth carrying out in a final package. According to this study, it is predictable that the product can maintain its quality over a long period and thus to produce a proper effect.

Acknowledgment

This work was supported by Gedeon Richter Ltd.—GINOP project (2.3.2-15-2016-00060) and The Ministry of Human Capacities, Hungary grant 20391-3/2018/FEKUSTRAT is also acknowledged.

References

1. H. Kublik, M.T. Vidgren, Nasal delivery systems and their effect on deposition and absorption, Advanced Drug Delivery Reviews. 1998;29:157-177. https://doi.org/10.1016/S0169-409X(97)00067-7.
2. U. Anand, T. Feridooni, R. U., Novel Mucoadhesive Polymers for Nasal Drug Delivery, in: A.D. Sezer (Ed.), Recent Advances in Novel Drug Carrier Systems, InTech, 2012. https://doi.org/10.5772/52560.
3. S.S. Chudiwal, M.H.G. Dehghan, Quality by design approach for development of suspension nasal spray products: a case study on budesonide nasal suspension, Drug Development and Industrial Pharmacy. 2016;42:1643-1652. https://doi.org/10.3109/03639045.2016.1160108.
4. R.P. Chen, From Nose to Brain: The Promise of Peptide Therapy for Alzheimer's Disease and Other Neurodegenerative Diseases, Journal of Alzheimer's Disease & Parkinsonism. 2017;07. https://doi.org/10.4172/2161-0460.1000314.
5. Y.S.R. Elnaggar, S.M. Etman, D.A. Abdelmonsif, O.Y. Abdallah, Intranasal Piperine-Loaded Chitosan Nanoparticles as Brain-Targeted Therapy in Alzheimer's Disease: Optimization, Biological Efficacy, and Potential Toxicity, Journal of Pharmaceutical Sciences. 2015;104:3544-3556. https://doi.org/10.1002/jps.24557.
6. E. Prommer, L. Thompson, Intranasal fentanyl for pain control: current status with a focus on patient considerations, Patient Preference and Adherence. 2011;157. https://doi.org/10.2147/PPA.S7665.
7. R. Narayan, M. Singh, O. Ranjan, Y. Nayak, S. Garg, G.V. Shavi, U.Y. Nayak, Development of risperidone liposomes for brain targeting through intranasal route, Life Sciences. 2016;163:38-45. https://doi.org/10.1016/j.lfs.2016.08.033.
8. A. Mistry, S. Stolnik, L. Illum, Nanoparticles for direct nose-to-brain delivery of drugs, International Journal of Pharmaceutics. 2009;379:146-157. https://doi.org/10.1016/j.ijpharm.2009.06.019.
9. P. Ruenraroengsak, J.M. Cook, A.T. Florence, Nanosystem drug targeting: Facing up to complex realities, Journal of Controlled Release. 2010;141:265-276. https://doi.org/10.1016/j.jconrel.2009.10.032.
10. C. Callens, J. Ceulemans, A. Ludwig, P. Foreman, J.P. Remon, Rheological study on mucoadhesivity of some nasal powder formulations, European Journal of Pharmaceutics and Biopharmaceutics. 2003;55:323-328. https://doi.org/10.1016/S0939-6411(03)00024-9.
11. A. Tanaka, T. Furubayashi, M. Tomisaki, M. Kawakami, S. Kimura, D. Inoue, K. Kusamori, H. Katsumi, T. Sakane, A. Yamamoto, Nasal drug absorption from powder formulations: The effect of three types of hydroxypropyl cellulose (HPC), European Journal of Pharmaceutical Sciences. 2017;96:284-289. https://doi.org/10.1016/j.ejps.2016.09.028.
12. Z.T. Al-Salama, L.J. Scott, Sumatriptan Nasal Powder: A Review in Acute Treatment of Migraine, Drugs. 2016;76:1477-1484. https://doi.org/10.1007/s40265-016-0641-9.
13. P. Gieszinger, I. Csóka, E. Pallagi, G. Katona, O. Jójárt-Laczkovich, P. Szabó-Révész, R. Ambrus, Preliminary study of nanonized lamotrigine containing products for nasal powder formulation, Drug Design, Development and Therapy. Volume 2017;11:2453-2466. https://doi.org/10.2147/DDDT.S138559.
14. P. Gieszinger, I. Tomuta, T. Casian, Cs. Bartos, P. Szabó-Révész, R. Ambrus, Definition and validation of the Design Space for co-milled nasal powder containing nanosized lamotrigine, Drug Development and Industrial Pharmacy. 2018;44:1622-1630. https://doi.org/10.1080/03639045.2018.1483388.
15. R. Ambrus, P. Gieszinger, R. Gáspár, A. Sztojkov-Ivanov, E. Ducza, Á. Márki, T. Janáky, F. Tömösi, G. Kecskeméti, P. Szabó-Révész, C. Bartos, Investigation of the Absorption of Nanosized lamotrigine Containing Nasal Powder via the Nasal Cavity, Molecules. 2020;25:1065. https://doi.org/10.3390/molecules25051065.
16. CPMP/ICH/380/95, Stability testing guidelines: Stability Testing of New Drug Substances and Products, (1993). http://www.pharma.gally.ch/ich/q1a038095en.pdf (accessed March 11, 2020).
17. M.D. Abràmoff, Image Processing with ImageJ, (n.d.) 7.


Hungarian Society for Pharmaceutical Sciences
Budapest, Established: 1924

Acta Pharmaceutica Hungarica moves to international level!

Acta Pharmaceutica Hungarica, the scientific journal of the Hungarian Society for Pharmaceutical Sciences will be published in English from 2019. The peer reviewed scientific journal will be published on-line in open access manner. Submission of original research papers (both full length and short communication) from all fields of pharmaceutical sciences are welcomed.

For further information and manuscript submission please visit the website of the journal:

www.aph-hsps.hu