ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIV, N 1, 12 SECTIO DDD 2011

[1] Dept. of Pharmacognosy with Medicinal Plant Laboratory, Medical University of Lublin, Poland
[2] Central Laboratory of Agroecology, University of Life Sciences in Lublin, Poland
[3] Dept. of Analysis and Evaluation of Food Quality, University of Life Sciences in Lublin, Poland
[4] Dept. of Virology, Medical University of Lublin, Poland

TOMASZ BAJ[1], ELWIRA SIENIAWSKA[1], RADOSŁAW KOWALSKI[2,3], ŁUKASZ ŚWIĄTEK[4], MAŁGORZATA MODZELELEWSKA[1], TADEUSZ WOLSKI[1]

Chemical composition and antioxidant activity of the essential oil of hyssop (Hyssopus officinalis L. ssp. officinalis). Part II. Free radical scavenging properties

Skład chemiczny oraz aktywność antyoksydacyjna olejku eterycznego z hyzopu lekarskiego (*Hyssopus officinalis L. ssp. officinalis*)
Część II. Właściwości przeciwwolnorodnikowe

INTRODUCTION

Essential oils obtained from various herbal organs differ in composition and possess different biological activity. Antibacterial, antifungal and antiviral properties are the most often described for the *Hyssopus officinalis* L. essential oil [17]. Mazzanti et al. [12] studied antibacterial activity of *H. officinalis* and *H. officinalis* var. *decumbens* essential oils. Agar diffusion test revealed that minimal inhibitory concentration (MIC) is similar for both samples. Both essential oils inhibited growth of *Staphyloccocus aureus* and yeast *Candida* (*albicans, crusei, tropicalis*). They were inactive against *Psedomonas aeruginosa*. The essential oil from *H. officinalis* var. *decumbens* inhibited *Escherichia coli Proteus mirabilis*, *Salmonella thypi* and *Salmonella typhi* growth better than *H. officinalis* essential oil. The assessment of the MIC and MBC values proved that *H. officinalis* var. *decumbens* essential oil is active against G „+", G „–" bacteria and yeast. Marino et al. [11] researched antimicrobial activity of the essentials oils from *Lamiaceae* and *Compositae* herbs. Sage, peppermint, chamomile, hyssop and oregano essential oils showed the highest activity in the concentration of 800 ppm after 60 minutes exposition time. In the hyssop essential oil pinocamphon, camphor i β–pinen were identified as the most active antimicrobial compounds. The hyssop essential oil has also insecticide activity. In the toxicity assay it caused mortality of 26.7% *Spodoptera litura* larvas [10] whereas methanolic extract form aerial parts of hyssop showed high activity against *Spodoptera littoralis* LD_{50} 1.78 (1.66–1.82) [16].

Pinocamphon and izo-pinocamphon, the main constituents of the hyssop essential oil, can be responsible for epileptogenic activity [19], De Vincenzi et al. [7] suggest that methyloeugenol can possess similar properties. The first reports about neurotoxic activity are from 1891, when the neurotoxic symptoms in the dog organism after 2.5 mg/kg dose were immediate [19]. Tisserand [19] described also several cases of overdosage of the hyssop essential oil when 15–30 drops taken by patients caused convulsions. Moreover, taking 10 drops per day also ended with epileptic attack, what suggest accumulation of the essential oil constituents in the human organism. Burkhard et al. [5] and Burfield [4] applied the hyssop essential oil in the tests on the rats. The 0.13 g/kg dose caused convulsions, whereas the same symptoms had been caused by 0.5 g/kg sage essential oil. Daily applications in the subclinical dose of 0.08g/kg lead to accumulation of the toxic effects. The exact mechanism of the neurotoxicity still needs a clarification and researchers recommend caution while using it during pregnancy [4]. The LD_{50} oral dose for a mouse equals 1.4 ml/kg, whereas LD_{50} transdermal dose for a rabbit is higher than 5 ml/kg [8,18].

Free oxygen radicals produced in the human cells can cause many diseases. Antioxidants are used to neutralise the oxidation and to trap free radicals [3,14]. Essential oil, containing major biologically active constituents of hyssop, is also a promising source of compounds possessing antioxidant properties. The increasing interest in antioxidants and free radicals justifies the need of evaluation of the antioxidant activity of the essential oil isolated from *Hyssopus officinalis* L.

This paper is a continuation of a previous research on a composition and biological activity of *Hyssopus officinalis* essential oil [2,20,21]. The stable radical DPPH in the methanol solution was used. EC_{50} was the appropriate parameter in the interpretation of this method (concentration at which the sample shows 50% radical-scavening activity). The antioxidant activity of the essential oil was compared to Trolox which is a standard antioxidant [3].

MATERIAL AND METHODS

Plant material, isolation of essential oil and GC/MS and GC/FID analysis conditions were described in previous publication [2].

TLC – DPPH antioxidant activity. The *H. officinalis* herb essential oil (OEH) was obtained in the Deryng-type apparatus according to the procedure described previously [2]. OEH was dissolved in the methanol (1:9) and applied (5, 10, 15 µl/spot) on the chromatographic plate Si60 G using autosampler ATS III (Camag, Switzerland). The plate was developed in the horizontal chamber DS-type (Chromdes, Poland) in the mixture of toluen and ethyl acetate (94:6 v/v). After development and air drying the plate was double derivatized. Part A was sprayed with 0.2% methanolic solution of DPPH, while part B with methanolic solution of vanilin in the sulphuric acid. Part B was heated in 15 s time using electic heater Bosh. The plate after derivatisation was placed in the dark for 30 min. The visulaisation of the results was done in the VIS-light using video-densitometr TLC Reprostar (Camag, Switzerland).

Antioxidant activity. The ability of essential oil to scaverage DPPH● (2.2-diphenyl-1-picrylhydrazyl-hydrate) free radical was assessed as described by Brand-Williams et al. [3]. The different concentrations of essential oils were tested (ranged from 5.68 mg/mL to 45.45 mg/mL) and then 0.1 mL was mixed with 3.9 mL of a $6x10^{-5}$ mol/L methanol DPPH solution. The mixture was incubated in dark at room temperature, the absorbance was measured in every 5 min. for 30 min. at 517 nm. The methanol was used as a blank instead of the extract. At the beginning deep violet color was observed and after 30 minutes it was light-yellow. Each kinetic reaction for different concentration was plotted spectrophotometrically. For visible absorbance measurements Cary Scan 50 (Varian, Inc, USA) was used. The radical scavenging activity of the essential oil was calculated from a calibration curve. The mean values were obtained from triplicate experiments. The oil concentration providing 50% inhibition (EC_{50} parameter) was calculated using following formula [3].

$$\% \text{ inhibition} = [(A_b - A_a)/A_b] \times 100$$

where, A_a is the absorbation of tested solution
A_b is the absorbation of blank sample

RESULTS AND DISCUSSION

The DPPH (2.2-difenylo-1-pikrylohydrazyl- free radical) assay is the one of the most often used for antioxidant activity evaluation. It can be applied to the antioxidant capacity determination in the fruits, juices, herbal extracts and food. This method is fast, accurate and reproducible. The alcoholic DPPH solutions are purple with the maximum absorbance λ=517 nm, stable in the ambient temperature. After neutralization DPPH solution turns yellow [6,9,13,15].

As a result of a screening TLC-DPPH *H. officinalis* essential oil analysis the number of active fractions was obtained. Fig. 1 presents developed and double derivatised chromatographic plate.

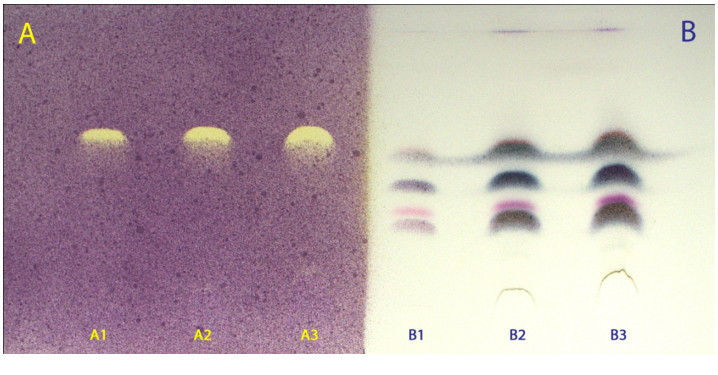

Fig. 1. Developed TLC plate with increasing amounts of applied essential oil 5µl (A1, B1), 10µl (A2, B2), 15µl (A3, B3), after derivatisation. Derivatisation: A – 0.2% methanolic solution of DPPH, B – methanolic solution of vanilin in sulphuric acid

As it can be seen from the double derivatised plate, on the part A DPPH radical was inhibited in the bright-yellow spots (R_f 0.52). Part B revealed six coloured bands with maximal R_f value 0.50 This results suggest that compounds having antioxidant activity are present in the essential oil. The comparison of the tracks after derivatisation (A2–green line; B2–black line) is shown on Fig. 2.

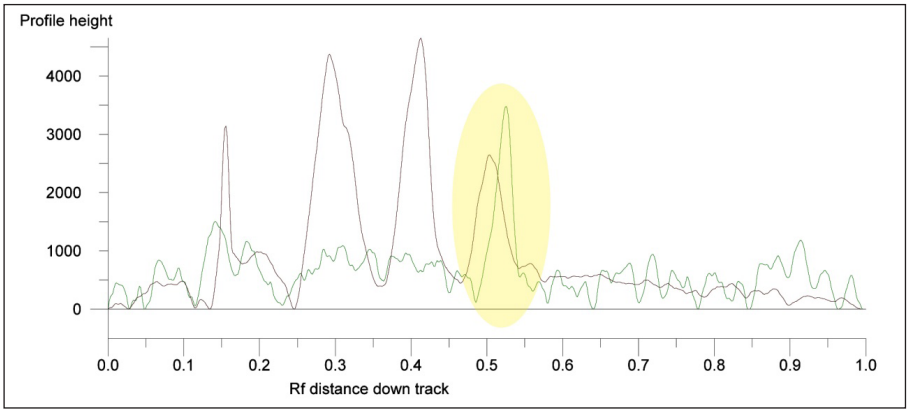

Fig. 2. The comparison of the developed tracks after derivatisation: green line – 0.2% methanolic solution of DPPH, black line – methanolic solution of vanilin in sulphuric acid

In the next step the antioxidant potential of the OEH in the DPPH assay was investigated. Kinetics reactions, percentage of inhibition and EC_{50} are shown in figures 3–5.

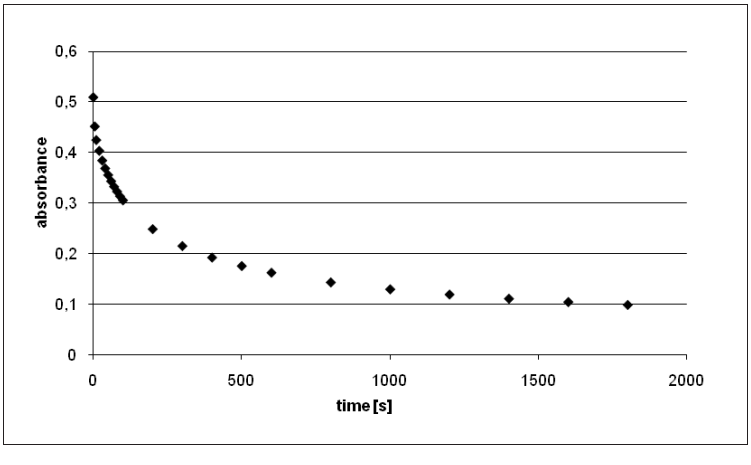

Fig. 3. Example of one kinetic behaviour of *Hyssopus officinalis* L.

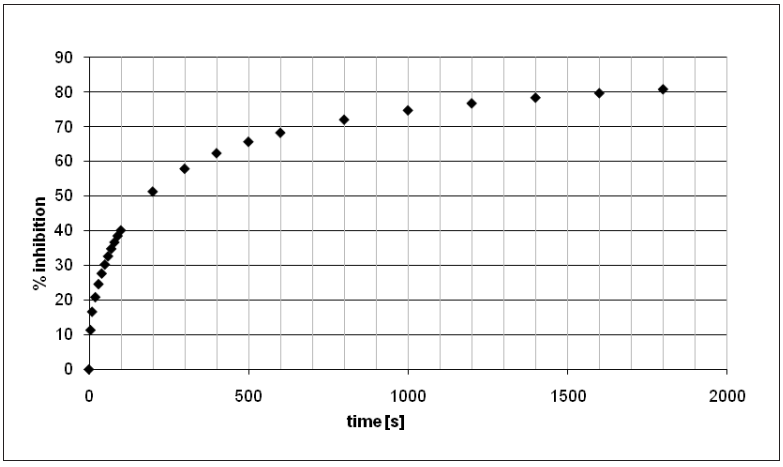

Fig. 4. Percentage of inhibition from kinetic reaction above

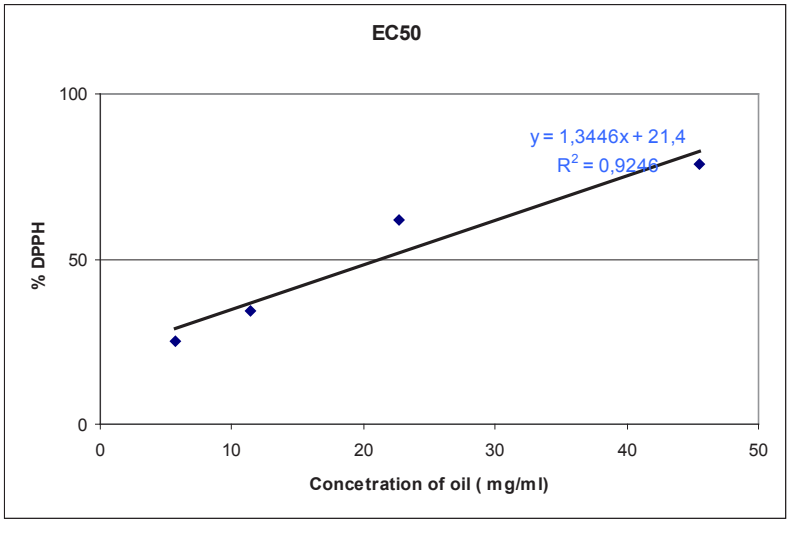

Fig. 5. Parameter EC_{50} of oil isolated from *Hyssopus officinalis* L.

The DPPH assay proved that Polish *H. officinalis* L. shows antioxidant properties. The EC_{50} parameter was 21.13 mg/mL whereas Trolox equivalent was 0.029 mM. According to Babovic et al. [1] hyssop aerial parts extract after SPE purification shows the weakest antioxidant activity comparing to rosemary, sage and thyme.

Because the essential oil composition is usually very complex the TLC analysis should be preliminary analysis. The components of the essential oil do not show fluorescence under UV light, therefore derivatisation with vanilin reagent is often needed. After spraying with this solution compounds separated on the chromatographic plate producing color bands visible after heating. This reaction shows the position of the main ingredient on the chromatographic plate. The DPPH test is used e.g. for evaluation of antioxidant properties of herbal extracts. Currentluy it is used in the TLC-bioautography tests [9]. Mimica-Dukić et al. [13] showed that TLC-DPPH method can be applied to the screening antioxidant tests of essential oils. Previous technics TLC-DPPH depended on the comparison of standards and investigated compounds R_f values obtained after derivatisation on the separated chromatographic plates. Parallel derivatisation on the one plate showed in this paper assures identical analysis parameters.

CONCLUSION

Application of the described parallel derivatisation technique on the same chromatographic plate revealed thet only one fraction of *H. officinalis* herb essential oil has an antioxidant potential. Double derivstiosation on the one plate assures identical analysis parameters and can be used for antioxidant-guided preparative isolation of active compounds.

SUMMARY

In the presented work parallel derivatisation with DPPH and Vanlin reagent on the same plate was shown. The investigated sample was essential oil obtained from *Hyssopus officinalis* L. aerial parts. Parallel derivatisation enabled identification of active, containing antioxidants fraction, which R_f was 0.52. The antioxidant activity of essential oil was examined against the stable free radical DPPH (2,2–diphenyl-1-picrylhydrazyl-hydrate). The antioxidant activity was expressed by using parameter EC_{50} and also compared with TEAC (Trolox). The results showed that the oil isolated from *Hyssopus officinalis L*. has low antioxidant activity EC_{50} value 21.13 mg/ mL and Trolox Equivalent value 0.029 mM.

Keywords: *Hyssopus officinalis L.*, hyssop, essential oil, DPPH, TLC-DPPH, antioxidant activity

STRESZCZENIE

W prezentowanej pracy zastosowano równoległą derywatyzację dwoma odczynnikami wywołującymi w celu określenia występowania grup składników odpowiadających za właściwości antyoksydacyjne olejku eterycznego z nadziemnych części hyzopu lekarskiego. Derywatyzacja 2% alkoholowym roztworem DPPH oraz alkoholowym roztworem waniliny w kwasie siarkowym pozwoliła na skriningowe określenie R_f frakcji związków antyoksydacyjnie czynnych. Analizowano aktywność antyoksydacyjną olejku przy użyciu odczynnika DPPH i przeliczono na ekwiwalent Troloxu. Olejek wykazywał niską aktywność, poziom EC_{50} wynosił 21,13 mg/ mL, zaś ekwiwalent Troloxu 0,029 mM.

Słowa kluczowe: *Hyssopus officinalis L.*, hyzop, olejek eteryczny, DPPH, TLC-DPPH, aktywność antyoksydacyjna.

REFERENCES

1. Babovic N., Djilas S., Jadranin M. et al.: Supercriticalcarbon dioxide extraction of antioxidant fractions from selected Lamiaceae herbs and their antioxidant capa city. Innov. Food Sci. Emerg. Techn., 11, 98, 2010.
2. Baj T., Kowalski R., Świątek Ł. et al.: Chemical composition and antioxidant activity of the essential oil of hyssop (*Hyssopus officinalis* L. ssp. *officinalis*). Annales UMCS, Sect. DDD, 3, 55, 2010.
3. Brand-Williams W., Berset C., Cuvelier M.E.: Use of a Free Radical Method to Evaluate Antioxidant Activity. Lebensm.-Wiss. Technol., 28, 25, 1995.
4. Burfield T.: Safety of essential oils. Int. J. Aromatherapy, 10, 16, 2000.
5. Burkhard P.R., Burkhard K., Haenggeli C.A., Landis T.: Plant-induced seizures: Reappearance of an old problem. J. Neurol., 246, 8, 667, 1999.
6. Cybul M., Nowak R.: Przegląd metod stosowanych w analizie właściwości antyoksydacyjnych wyciągów roślinnych. Herba Pol. 1, 69, 2008. [In Polish]
7. De Vincenzi M., Silano M., Stacchini P., Scazzocchio B.: Constituents of aromatic plats: I. Methyleugenol. Fitoter., 71, 216, 2000.
8. Góra J., Lis A.: Najcenniejsze olejki eteryczne. Wyd. UMK, Toruń, 2004. [In Polish]
9. Gu L., Wu T., Wang Z.: TLC bioautography-guided isolation of antioxidants from fruit of Perilla frutescens var. acuta. Food Sci Techn., 42, 131, 2009.
10. Isman M.B., Wan A.J., Passreiter C. M.: Insecticidal activity of essential oils to the tobacco cutworm, *Spodoptera litura*. Fitoter., 72, 65, 2001.
11. Marino M., Bersani C., Comi G.: Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. Int. J. Food Microbiol., 67, 187, 2001.
12. Mazzanti G., Battinelli L., Salvatore G.: Antibacterial properties of the linalol-rich essentials oil of *Hyssopus officinalis* L. var. decumbens (*Lamiaceae*). Flavour Fragr. J., 13, 289, 1998.
13. Mimica-Dukić N., Bugarin D., Grabović S. et al.: Essential oil of *Myrtus communis* L. as a potential antioxidant and antimutagenic agents. Molecules, 15, 2759, 2010.
14. Mensor L.L., Menezes F.S., Leitao G.G. el al.: Screening of Brazilian Plant Extracts for Antioxidant Activity by the Use of DPPH Free Radical Method. Phytother. Res., 15, 127, 2001
15. Nowak R., Gawlik-Dziki U.: Polyphenols of *Rosa* L. leaves extracts and their Radical Scavening Activity. Z. Naturforsch. C, 62, 32, 2007.
16. Pavela R.: Insecticidal activity of certain medicinal plants. Fitoter., 75, 745, 2004.
17. Piccagalia R., Tammaro F., Pace L.: Characterization of essential oils from three Italian ecotypes of hyssop (*Hyssopus officinalis* L. subsp. *aristatus* Gordon Briq.). J. Essent. Oil Res., 11, 693, 1999.
18. Poppenga R.H.: Herbal medicine: potential for intoxication and interactions with conventional drugs. Clin. Techn. Small Anim. Pract., 17, 1, 6, 2002.
19. Tisserand R.: Essential oil safety II. Int. J. Aromatherapy, 7, 4, 26, 1996.
20. Wolski T., Baj T., Kwiatkowski S.: Hyssop (*Hyssopus officinalis* L.) forgotten medicinal, flavoring and honey-yields plant. Annales UMCS, Sect. DD, 61, 1, 2006.
21. Wolski T., Baj T.: Hyzop lekarski (*Hyssopus officinalis* L.) – aromatyczna roślina lecznicza. Aromaterapia, 12, 10, 2006. [In Polish]

ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIV, N 1, 13 SECTIO DDD 2011

Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Poland

ANTONINA MAZUR, SYLWIA FIDECKA

The antinociceptive effects of topiramate evaluated in writhing test in mice

Ocena antynocyceptywnych efektów topiramatu w teście przeciągania u myszy

INTRODUCTION

Topiramate (TPM) is a newer generation antiepileptic drug used in the treatment of partial-onset and primary generalized tonic-clonic seizure, and also Lennox-Gastaut syndrome in patients as young as 2 years in adjunctive therapy [12]. Chemically, it is a monosaccharide – D-fructose derivative with a sulfamate functionality which distinguishes it from other antiepileptic drugs (AEDs) [21].

TPM reveals multiple mechanism of action which includes: 1) voltage-dependent sodium channel blockade and modulation 2) enhancement of GABA receptor–mediated inhibition (however, it is noteworthy that TPM does not bind the benzodiazepine place on the GABA receptor complex), 3) antagonism of excitatory transmission represented by antagonistic effect at AMPA (but not NMDA) receptor sites [16,21] 4) some isozymes of carbonic anhydrase inhibition (especially CA-II and CA-IV) and 5) negative modulation of voltage-gated calcium ion channels. Modulation of K^+ conductance and proteins regulating neurotransmitter release from synaptic terminals has also been proposed as potential mechanism of action of TPM [22].

A variety of pharmacodynamic properties of TPM suggests potential efficacy in conditions other than epilepsy. Additionally, TPM possesses neuronal stabilising properties which may be beneficial in the treatment of pain [28].

Recently, newer AEDs are used by choice in the treatment of non-epileptic pathologies e.g. pain conditions [2,7]. Some of them are first choice drugs used in the prophylaxis of headache and also belong to the group of adjunctive drugs for various types of neuropathic pain [13]. It is the result of their exceptional mechanism of action, better tolerability and better pharmacokinetic profiles compared to standard drugs used in neuropathic pain and migraines such as tricyclic antidepressants or conventional AEDs [20].

Large controlled studies have assessed the efficacy of TPM in migraine prevention. Therefore, it is accepted for migraine prophylaxis in numerous countries throughout the world [24]. Numerous studies have proven that TPM reduces intensity, duration and frequency of migraine appearance [20,22] and improves the quality of life in migraine patients [3,22]. Moreover, it has been suggested

that TPM reduces the risk of transforming episodic headache to chronic form of headache [22] and even helps in reversion of chronic migraine to episodic one [5]. Some other data point to therapeutic clinical benefits of TPM in the management of chronic migraine, basilar migraine and vestibular migraine, cluster headache [2] and what is worth emphasizing in pediatric migraine [2,8,12].

TPM influences the central and peripheral nervous system, which can lead to changes in pain producing processes. This phenomenon allowed presuming efficacy of TPM in neuropathic pain i.e. diabetic neuropathy, trigeminal neuralgia [4,6,11]. Unfortunately, the discrepancy between the results of number of studies has been observed [7,20,28] and some authors suggest that TPM may not be effective in the treatment of neuropathic pain. Some pilot studies have also pointed to efficacy of TPM in several psychiatric conditions, including alcohol dependence, binge-eating disorder, bulimia nervosa, posttraumatic stress disorder [24] and also in difficult-to-treat conditions associated with medication overuse [22].

As it has been mentioned above, many AEDs are widely investigated by scientists for their potential analgesic properties. Literature data concerning effectiveness of TPM in pain is incomplete and controversial. Therefore, the aim of present paper is to study the antinociceptive activity of TPM, both given alone and in combination with other substances with confirmed analgesic properties in the writhing test in mice.

MATERIALS AND METHODS

Animals. Experiments were carried out on male Albino Swiss mice (18–30 g). Animals were kept in 8–10 to a cage at room temperature of 20 ± 1 ºC and 12 h light/dark cycle. Standard food (Murigran pellets, Bacutil, Motycz, Poland) and water were available *ad libitum* with exception of experiments when food was taken away. All experiments were performed between 9:00 a.m. and 3:00 p.m. Animals were acclimatized to the experimental room for about 2 h before testing, were used only once and sacrificed immediately after the test with a lethal dose of gaseous carbon dioxide (CO_2).

All behavioral experiments were carried out according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and to the European Community Directive for the Care and Use of Laboratory Animals of 24 November 1986 (86/609/EEC), and approved by the Local Ethics Committee.

Drugs. The following drugs were used: topiramate (Topamax, Janssen-Cilag, Belgium), naloxone hydrochloride (Sigma, USA), morphine hydrochloride (Polfa, Poland), ethanol (Polmos, Poland), diazepam (Relanium, Polfa, Poland), ketamine (Ketanest, Parke-Davis, Germany).

The writhing test. The nociceptive responses in mice were investigated in the writhing test according to Koster et al. [15]. Each mouse received one intraperitoneal (*ip*) injection of 10 mg/kg of 0.6% acetic acid solution to evoke writhing. The mice were placed singly in a glass cylinder (35 cm high, 25 cm in diameter) and the number of abdominal constrictions (writhing episodes) was counted during a 10 min period, starting 5 min after the acetic acid administration. TPM was injected subcutaneously (*sc*), as a suspension in 0.5 % tylose solution, 60 min before the test. The other drugs (excluding ethanol, which was given intragastrically (*po*), 15 min before acetic acid) were injected *sc*: 10 min (naloxone and diazepam), 20 min (morphine) and 30 min (ketamine) before the acid. The absolute mean values of writhing episodes in control groups ranged from 16.80 ± 3.323 to 30 ± 5.018 (mean ± SEM) and were shown as 100%. The equivalent volume of vehicle (tylose) and 0.6% acetic acid solution was administered to the control groups.

Statistical analysis. The obtained data have been analyzed by using One-way ANOVA analysis of variance. Post hoc comparisons were carried out by Tukey-Kramer test. P values < 0.05 and lower have been considered as statistically significant.

RESULTS

TPM administered at the doses of 25, 50 and 100 mg/kg (*sc*) (Fig.1) significantly ($p < 0.01$, $p < 0.05$ and $p < 0.05$, respectively) decreased the number of writhing episodes when compared to vehicle. The slight, nonsignificant effect was observed after the administration of TPM at the dose of 12.5 mg/kg which was accepted as the threshold dose (Fig.1). Our unpublished data have shown that the strongest effect of TPM (25 mg/kg, *sc*) reveals 60 min after the administration, therefore the writhing test was conducted 60 min after injection of the anticonvulsant.

Naloxone (5 mg/kg, *sc*) was able to reverse ($p < 0.05$) antinociceptive effects of TPM (25 mg/kg) (Fig.2).

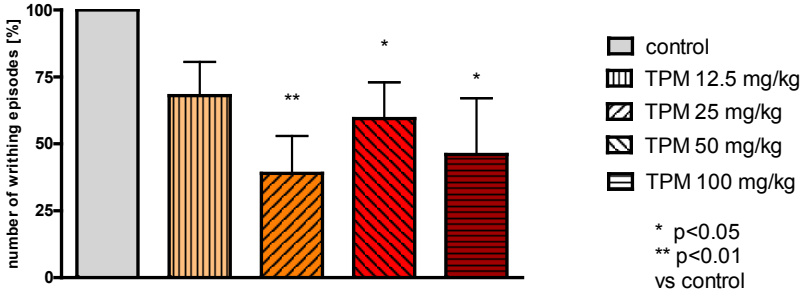

Fig. 1. The antinociceptive activity of various doses of topiramate (TPM) assessed in the writhing test in mice. Each bar represents the mean ± SEM for a group of 8–10 mice. The data are expressed as per cent of control group. * $p < 0.05$, ** $p < 0.01$ vs control group (one-way ANOVA test)

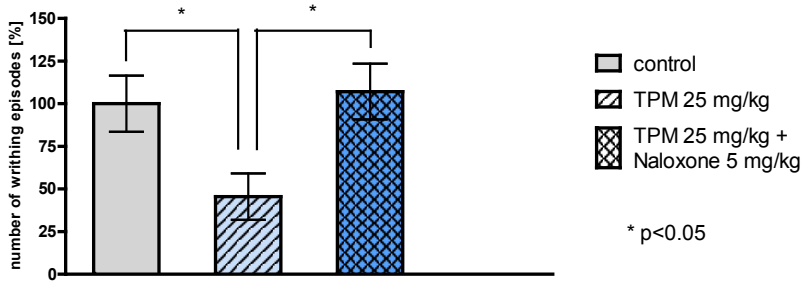

Fig. 2. The influence of naloxone on antinociceptive activity of topiramate (TPM) assessed in the writhing test in mice. The results are expressed as means ± SEM for a group of 8–10 mice. The mean value of a number of writhing episodes in the control group was assumed to be 100 %. * $p < 0.05$ (one-way ANOVA test)

Co-administration of TPM (12.5 mg/kg) and ethanol (1 g/kg) (both administered at the threshold doses) has resulted in antinociception observed as a reduction in writhing response. This effect was significant ($p<0.05$) in comparison to TPM group but not ethanol group (Fig.3).

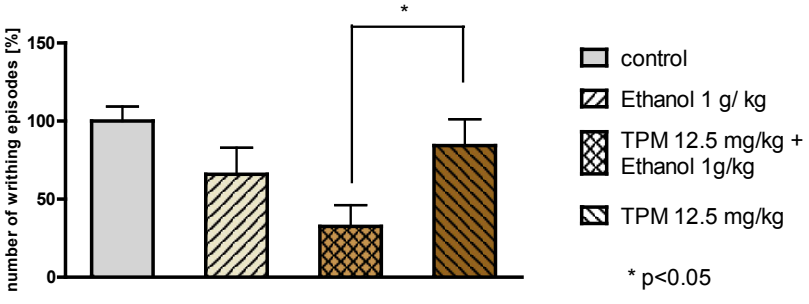

Fig. 3. The influence of topiramate (TPM) on antinociceptive activity of ethanol (given at the threshold doses) assessed in the writhing test in mice. The results are expressed as means ± SEM of group consisting of 8–10 mice. The mean value of a number of writhing episodes in the control group was assumed to be 100 %. * $p < 0.05$ (one-way ANOVA test)

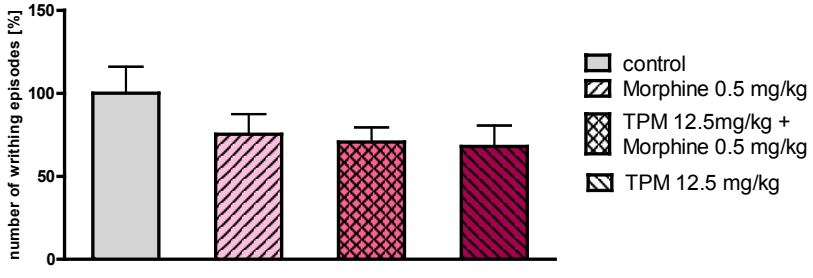

Fig. 4. The influence of topiramate (TPM) on antinociceptive activity of morphine (given at the threshold doses) assessed in the writhing test in mice. The results are expressed as means ± SEM of group consisting of 8–10 mice. The mean value of a number of writhing episodes in the control group was assumed to be 100 % (one-way ANOVA test)

The lack of co-operation was noted when TPM (12.5 mg/kg) was associated with one of the drugs: morphine (0.5 mg/kg) (Fig.4), diazepam (1.25 mg/kg) (Fig.5) or ketamine (10 mg/kg) (Fig 6). All substances given alone at the subthreshold doses did not decrease or slightly decreased the number of writhing episodes in mice (respectively Fig.4, 5 and 6).

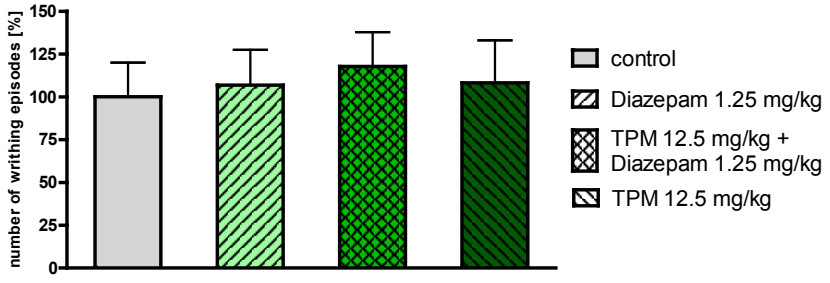

Fig. 5. The influence of topiramate (TPM) on antinociceptive activity of diazepam (given at the threshold doses) assessed in the writhing test in mice. The results are expressed as means ± SEM of group consisting of 8–10 mice. The mean value of a number of writhing episodes in the control group was assumed to be 100 % (one-way ANOVA test)

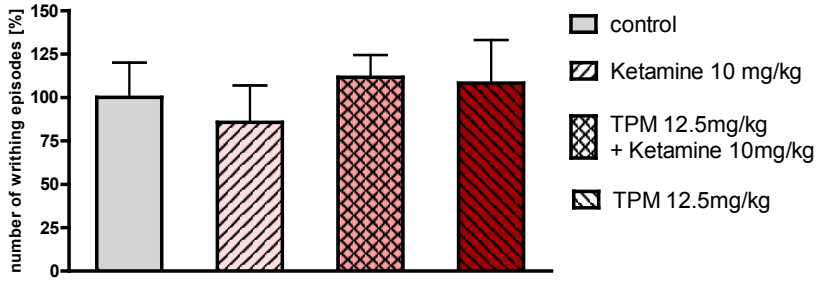

Fig. 6. The influence of topiramate (TPM) on antinociceptive activity of ketamine (given at the threshold doses) assessed in the writhing test in mice. The results are expressed as means ± SEM of group consisting of 8–10 mice. The mean value of a number of writhing episodes in the control group was assumed to be 100 % (one-way ANOVA test)

DISCUSSION

Many of the putative mechanisms of action that have caused the newer AEDs effective anti-seizure medications might also allow them to alleviate the pain [20]. Based on the hypothesis that epilepsy shares together with migraine and neuropathic pain several pathogenetic mechanisms, some AEDs are used in the prevention of migraine and other types of pain [1,20]. Imbalance between excitatory glutamate-mediated transmission and GABA-mediated inhibition in specific brain areas and lower threshold for the induction of long-term changes in neuronal excitability (sensitization and kindling) both in epilepsy and migraine, have been postulated [1]. Moreover, abnormal activation of voltage-operated ionic channels has been implicated in these two pathological conditions. Also cortical spreading depression has been found to be involved in both pathophysiology of epilepsy and generation of migraine aura [1,22].

The presumable mechanism of action of AEDs in migraine is probably due to decreasing brain excitability, as well as increasing the threshold for activation in the brainstem areas important for initiating migraine [2]. It is essential ability, especially that migraine and epilepsy are comorbid conditions: epilepsy occurs more commonly in patients with migraine than in general population, and *vice versa*, migraine in epileptics [1].

The analgesic properties of TPM have been analyzed in various models of pain in animals what has shed some light on mechanisms involved in observed activity.

Some studies have pointed to TPM's analgesic action in various models of neuropathic pain. Among others, its antiallodynic effect in both Chung and Seltzer models of neuropathic pain was observed in rats [30].

The efficacy of TPM in acute pain tests has also been evaluated. Lopes et al. [18] have reported an effectiveness of TPM in formalin and hot plate tests. They have found that TPM's analgesic action was reversed by naloxone (2 mg/kg, s.c.), which would suggest that the opioid system may participate in the observed effects, whereas the activation of ATP-dependent potassium channels or serotoninergic system via 5HT2A and 5HT3 receptors is not involved in mechanism of antinociceptive effects of TPM [18]. However, other authors have not shown the activity of TPM in formalin test in rats [23]. This discrepancy may be explained by the use of a higher dose of anticonvulsant by Lopes et al. [18], compared to smaller doses and different animal species used in other studies.

In the present study it has been decided to choose writhing test which is a model of visceral pain. In this test both peripheral and central analgesia effects are estimated. Many investigators have used it and recommended as a simple and very sensitive screening method. Furthermore, a good correlation between the potencies of analgesics in writhing test in animals and their clinical potencies has been noted [17,29].

The writhing test has already been used to evaluate analgesic properties of TPM by Stepanović et al. [25]. However, different route of administration (*sc*) and higher doses of TPM were used in our experiments. Moreover, the antinociceptive action of TPM in combination with morphine, ethanol, ketamine and diazepam has been additionally analyzed in our study.

In the present study, it has been shown that TPM, given at the doses of 25, 50 and 100 mg/kg (*sc*), was able to induce antinociceptive action in writhing test in mice, and maximal intensity of observed effect has been noticed 60 min after administration of the drug. The described activity is not dose-dependent but rather limited, because of the similarity of antinociceptive power of three effective doses. The power of TPM-induced antinociception is comparable to that of 1 mg/kg of morphine observed in the writhing test in mice [19]. These results are partially in agreement with these of Stepanović et al. [25] in which even lower dose of TPM (10 mg/kg) than these used in our study had antinociceptive activity. This discrepancy may be also the result of different route of TPM's administration – *sc* and *po*. Moreover, antinociceptive activity of TPM was not the result of potent motor disturbing effect or sedation because it did not alter motor performance even at high doses (400–1500 mg/kg, *po*), for up to 2 h of observation in mice [25].

The antinociceptive effect of TPM (25 mg/kg) was significantly attenuated by naloxone (5 mg/kg) which is consistent with findings presented by Lopes et al. [18]. It should be noted that naloxone given alone at this dose did not alter the response of mice to chemical nociceptive stimuli in the writhing

test [26]. Naloxone is μ-, κ- and δ-opioid receptor competitive antagonist with especially high affinity for μ-opioid receptor [14]. Activation of the μ- receptor by an agonist causes, among others, strong analgesia. δ-opioid receptor is also thought to play a role in analgesia [14]. Analgesic effects of such opioid agonists as morphine are antagonized by naloxone. Ability of naloxone to diminish the antinociception of TPM seems to suggest connection with the endogenous opioid system. However, it is essential to note that this dose of naloxone may affect not only opioid receptors. On the other hand, the observed lack of synergism of TPM with morphine in writhing test puts into question above suggestion and seems to eliminate at least μ-opioid receptors as a target of TPM's antinociceptive action. Thus, the noticed differences may be a result of the affinity to δ-opioid receptors presented by naloxone and the lack of such affinity in a case of morphine.

Ethanol exerts strong effect, especially on the central nervous system, through disturbing the balance between excitatory and inhibitory influences in the brain. Although it does not have its own receptor [27], general literature data points to the $GABA_A$ receptor as an important target for the activity of ethanol [10]. The antinociceptive effects of ethanol are well known. The ethanol-produced antinociception and the developement of tolerance to this effect as a result of its chronic administration in mice and rats were confirmed by Fidecka et al. [9]. In connection with the influence of ethanol on δ-opioid receptors, it is supposed that the opioid system participates in its antinociceptive effects [9]. The antinociceptive effects of threshold dose of TPM were significantly enhanced by ethanol which could be a result of their common influence on GABA and/or hyperexcitable aminoacids systems. Moreover, in light of the mentioned above partial conclusions, it may also point to participation of the opioid system via δ-opioid receptors in observed effects.

The interactions between TPM and ketamine or diazepam have not been shown. Therefore, the participation of GABA and hyperexcitable aminoacids systems seems to be debatable and further studies are required to precisely determine the role of both these systems in TPM's mechanism of action.

At the end, it should be underlined that anticonvulsant and analgesic effects of AEDs do not necessarily correlate and therefore the anticonvulsant and analgesic activity may be due to separate, unrelated mechanisms [23].

In conclusion, our studies confirm the effectiveness of TPM in writhing test in mice and seem to suggest that the opioid system, at least partially, participates in the analgesic mechanism of action of the drug. However, the mechanism of the analgesic action of TPM has not been yet fully delineated and further investigations in this area are needed.

REFERENCES:

1. Calabresi P. et al.: Antiepileptic drugs in migraine: from clinical aspects to cellular mechanisms. Trends Pharmacol. Sci., 28, 188, 2007.
2. Carmona S., Bruera O.: Prophylactic treatment of migraine and migraine clinical variants with topiramate: an update. Ther. Clin. Risk Manag., 5, 661, 2009.
3. Dahlöf C. et al.: The impact of migraine prevention on daily activities: a longitudinal and responder analysis from three topiramate placebo-controlled clinical trials. Health Qual Life Outcomes, 5, 56, 2007.

4. Donofrio P.D. et al.: Safety and effectiveness of topiramate for the managament of painful diabetic peripheral neuropathy in an open-label extension study. Clin. Ther., 27, 1420, 2005.
5. Edvinsson L., Linde M.: New drugs in migraine treatment and prophylaxis: telcagepant and topiramate. New Drugs Class, The Lancet, 376, 645, 2010.
6. Edwards K.R. et al.: Efficacy and safety of topiramate in the treatment of painful diabetic neuropathy: A double-blind, placebo-controlled study. Neurology, 54 (Suppl 3): A81, Abstract, 2000.
7. Ferrante F.M.: The pharmacologic managament of neuropathic pain. Semin Anesth, Perioperative Medicine and Pain, 22, 168, 2003.
8. Ferraro D., Di Trapani G.: Topiramate in the prevention of pediatric migraine: literature review. J. Headache Pain, 9, 147, 2008.
9. Fidecka S. et al.: The development of cross tolerance between ethanol and morphine. Pol. J. Pharmacol. Pharm., 38, 277, 1986.
10. Fleming M., Mihic S.J., Harris R.A.: Ethanol. In: Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 11th edn. Eds. Brunton LL, Lazo JS, Parker KL, McGraw-Hill, USA, 591, 2006.
11. Gilron I. et al.: Topiramate in trigeminal neuralgia: a randomized, placebo-controlled multiple crossover pilot study. Clin Neuropharmacol, 24, 109, 2001.
12. Golden A.S., Sheryl R.H., Solomon L.M.: Nonepileptic uses of antiepileptic drugs in children and adolescents. Pediatr Neurol, 34, 421, 2006.
13. Guay D.R.: Adjunctive agents in the management of chronic pain. Pharmacotherapy, 21, 1070, 2001.
14. Gutstein H.B., Akil H.: Opioid analgesics. In: Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 11th edn. Eds. Brunton LL, Lazo JS, Parker KL, McGraw-Hill, USA, 547, 2006.
15. Koster R., Anderson M., De Beer E.J.: Acetic acid for analgesic screening. Fed. Proc., 18, 412, 1959.
16. Langtry H.D., Gillis J.C., Davis R.: Topiramate. A review of its pharmacodynamic and pharmacokinetic properties and clinical efficacy in the managament of epilepsy. Drugs, 54, 752, 1997.
17. Le Bars D., Gozariu M., Cadden S.W.: Animal models of nociception. Pharmacol. Rev., 53, 597, 2001.
18. Lopes L.S.: Antinociceptive effect of topiramate in models of acute pain and diabetic neuropathy in rodents. Life Sci., 84, 105, 2009.
19. Matosiuk D.: Synthesis and pharmacological activity of new carbonyl derivatives of 1-aryl-2-iminoimidazolidine. Part 2. Synthesis and pharmacological activity of 1,6-diaryl-5,7(1*H*)dioxo-2,3-dihydrimidazo[1,2-*a*][1,3,5]triazines. Eur. J. Med. Chem., 37, 761, 2002.
20. Pappagallo M.: Newer antiepileptic drugs: possible uses in the treatment of neuropathic pain and migraine. Clin. Ther., 25, 2506, 2003.
21. Rosenfeld W.E.: Topiramate: a review of preclinical, pharmacokinetic, and clinical data. Clin Ther, 19, 1294, 1997.
22. Ruiz L.: Topiramate in migraine progression. J Headache Pain, 2009, 10, 419, 2009.
23. Shannon H.E., Eberle E.L., Peters S.C.: Comparison of the effects of anticonvulsant drugs with diverse mechanisms of action in the formalin test in rats. Neuropharmacology, 48, 1012, 2005.
24. Silberstein S.D.: Topiramate monotherapy in epilepsy and migraine prevention. Clin Ther, 27, 154, 2005.
25. Stepanović-Petrović R.M.: The antinociceptive effects of anticonvulsants in a mouse visceral pain model. Anesth. Analg., 106, 1897, 2008.

26. Talarek S., Fidecka S.: Role of nitric oxide in benzodiazepi es-induced antinociception in mice. Pol. J. Pharmacol. 54, 27, 2002.
27. Tabakoff B., Hoffman P.: Biochemical pharmacology of alcohol. In: Psychopharmacology. Ed. Meltzer H.Y., The Third Generation of Progress. Raven Press, New York, 1521, 1987.
28. Thienel U.: Topiramate in painful diabetic polyneuropathy: findings from three double-blind placebo-controlled trials. Acta. Neurol. Scand., 110, 221, 2004.
29. Vogel G.H., Vogel W.H.: Drug Discovery and Evaluation, Pharmacological Assays Springer-Verlag, Berlin, 382, 1997.
30. Wieczorkiewicz-Płaza A.: Effect of topiramate on mechanical allodynia in neuropathic pain model in rats. Pol. J. Pharmacol., 56, 275, 2004.

SUMMARY

In the present study the antinociceptive activity of topiramate (TPM), new antiepileptic drug with multiple mechanism of action, in writhing test in mice was evaluated. It has been shown that TPM, given at the doses of 25, 50 and 100 mg/kg, was able to induce antinociception.

The diminution of TPM's antinociception by naloxone and the lack of interactions between TPM and morphine, suggest that δ-opioid receptors are engaged in observed antinociceptive effects of TPM. Moreover, the antinociceptive effects of threshold dose of TPM were significantly enhanced by ethanol which could be a result of their common influence on GABA and/or hyperexcitable aminoacids systems and may also point to participation of δ-opioid receptors. The interactions between TPM and ketamine or diazepam have not been shown. Therefore, the role of GABA and hyperexcitable aminoacids systems in mechanism of action of TPM seems to be debatable. In conclusion, our studies confirm the effectiveness of TPM in writhing test in mice and seem to suggest that opioid system, at least partially, participates in analgesic mechanism of action of the drug. However, further investigations in this area are needed.

Keywords: topiramate, antinociception, writhing test, mice.

STRESZCZENIE

W prezentowanej pracy oceniano antynocyceptywną aktywność topiramatu (TPM), leku przeciwpadaczkowego nowej generacji o złożonym mechanizmie działania, w teście przeciągania u myszy. Wykazano, iż TPM podawany w dawkach 25, 50 i 100 mg/kg wykazywał właściwości antynocyceptywne. Zmniejszenie aktywności antynocyceptywnej TPM przez nalokson, jak również brak interakcji pomiędzy TPM a morfiną, sugeruje, że receptory δ-opioidowe są zaangażowane w obserwowane efekty TPM. Dodatkowo antynocyceptywne działanie progowej dawki TPM było istotnie nasilane przez etanol, co może świadczyć o wpływie obydwu substancji na układ GABA-ergiczny i/lub aminokwasów pobudzających, jak również może wskazywać na udział w obserwowanych receptorów δ-opioidowych. Jakkolwiek, brak interakcji pomiędzy TPM a ketaminą lub diazepamem wydaje się podważać rolę układu GABA-ergicznego i układu aminokwasów pobudzających w mechanizmie działania TPM.

Podsumowując, nasze badania potwierdzają skuteczność TPM w teście przeciągania u myszy i wydają się sugerować przynajmniej częściowy udział układu opioidowego w przeciwbólowym mechanizmie działania tego leku. Niemniej jednak, kolejne badania w tym kierunku są wymagane

Słowa kluczowe: Topiramat, antynocycepcja, test przeciągania, myszy