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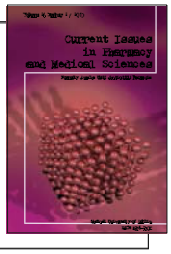

Effects of various naturally occurring compounds (arbutin, borneol, esculetin, esculin, ellagic acid, gallic acid, hesperidine, piperitol, piperonal, quercetin, thymoquinone and ursolic acid) against maximal electroshock-induced seizures in mice

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ABSTRACT

The aim of this study was to perform the anticonvulsant screening test to select some naturally occurring substances isolated from herbs and medicinal plants that could offer a distinct protection against maximal electroshock (MES)-induced tonic seizures in mice. The screening test was performed for 12 substances (i.e., arbutin, borneol, esculetin, esculin, ellagic acid, gallic acid, hesperidine, piperitol, piperonal, quercetin, thymoquinone and ursolic acid) administered intraperitoneally in a constant dose of 300 mg/kg at various pretreatment times (i.e., 15, 30, 60 and 120 min.) before the MES test. Results indicate that only borneol produced a 75% protection against MES-induced tonic seizures in mice, when administered i.p. at 15 min. prior to the MES test. Borneol administered i.p. at 30 min before the MES test protected a 37.5% of animals tested, whereas the compound administered i.p. at 60 min. prior to the test exerted barely a 12.5% protection against MES-induced tonic seizures. In contrast, borneol administered i.p. at 120 min. prior to the test produced no anticonvulsant activity in mice subjected to the MES test. The remaining substances tested in the mouse MES model (i.e., arbutin, esculetin, esculin, ellagic acid, gallic acid, hesperidine, piperitol, piperonal, quercetin and ursolic acid) exerted no or negligible anti-seizure activity after their i.p. administration to mice. In conclusion, borneol is worthy of consideration as a potentially favorable compound in epileptology, if the results from this study could be extrapolated into clinical settings.

Keywords: arbutin, borneol, esculetin, esculin, ellagic acid, gallic acid, hesperidine, piperitol, piperonal, quercetin, thymoquinone, ursolic acid, maximal electroshock seizure test

INTRODUCTION

Despite advanced knowledge on pathophysiological processes underlying epileptogenesis and several classical, second- and third-generation antiepileptic drugs (AEDs) currently available in clinical practice, there is still approx. 30% of epileptic patients resistant to these AEDs applied in monotherapy [7, 8]. In such a situation, epileptic patients require polytherapy with two or more AEDs or the application of some novel AEDs, as adjunctive drugs added to classical AEDs to stop seizure attacks. Nowadays, researchers and clinicians are trying to find some novel AEDs among various naturally occurring substances isolated from herbs and medicinal plants that could suppress seizures in patients with refractory epilepsy.

Traditional knowledge obtained from ancient medical literature or from folkloric medicine can play an important role in developing new drugs. There is no doubt that medicinal plants have played an invaluable role in the drug discovery process [1, 4-6]. For instance, several plants that were reputed to possess antiepileptic properties in different folklore cultures have been found to exhibit anticonvulsant activity in different animal models [20].

Relatively recently, there has appeared a trend in pharmaceutical studies to evaluate the anticonvulsant activity of essential oils obtained from medicinal plants and vari-

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ous herbs. For instance, it has been documented that the essential oils isolated from Artemisia dracunculus, Eugenia caryophyllata, Pimpinella anisum, Ascorus tatarinowii, Cymbopogon citratus, Bunium persicum, Ferula gummosa and Zhumeria majdae exerted the anticonvulsant activity in the maximal electroshock (MES)-induced seizure model in mice [2, 9, 16-19, 21, 22, 24]. In all cases, the essential oils that suppressed MES-induced tonic seizures underwent subsequent analytical investigation and researchers were looking for the most active compounds. It seems that the search for the anticonvulsant agents among compounds identified in essential oils is a novel approach in experimental epileptology. No doubt exists that the active compounds that could be isolated from plants would become an important source for the development of better and safer drugs for the treatment of epilepsy. In such a situation, experimental animal studies are required to perform the screening test allowing the identification of the active compounds naturally occurring in herbs and medicinal plants.

In our earlier studies, we have reported that imperatorin, osthole and xanthotoxin (three naturally occurring coumarins) produced the anticonvulsant action in the mouse MES model [11-15]. In contrast, two other coumarins, bergapten and oxypeucedanin had no impact on MES-induced tonic seizures in mice [11].

Considering the above-mentioned facts, we sought to perform the first anticonvulsant screening test to select some substances with anticonvulsant properties in the mouse MES model. This animal model allows selecting agents possessing the anticonvulsant activity because in this test, several classical, second- and third-generation AEDs (including, carbamazepine, phenytoin, phenobarbital, valproate, lamotrigine, oxcarbazepine, lacosamide, retigabine, pregabalin and topiramate) exert the anticonvulsant action by suppressing tonic seizures in mice [10]. Since these AEDs are clinically efficacious in patients with tonic-clonic seizures and partial convulsions with or without secondary generalization [3], and suppressed tonic seizures in the MES test in rodents, it was appropriate to use this experimental model as a first screening test allowing for the selection of compounds with anticonvulsant properties. It is important to note that seizure models in laboratory animals are still the most important tools in preclinical search for agents and compounds possessing the anticonvulsant activity.

Therefore, in this study we tested the anticonvulsant action of arbutin, borneol, esculetin, esculin, ellagic acid, gallic acid, hesperidine, piperitol, piperonal, quercetin, thymoquinone and ursolic acid against MES-induced tonic seizures in mice. All these 12 naturally occurring substances were isolated from various herbs and medicinal plants growing in Poland and these compounds belong to various diverse chemical families, including, coumarins, glycosylated hydroquinones, flavonoids, monoterpenes and triterpenes.

MATERIALS AND METHODS

Animals and experimental conditions. Adult male Swiss mice (weighing 22-26 g) that were kept in colony cages with free access to food and tap water, under standardized housing conditions (natural light-dark cycle, temperature of 23 ± 1°C, relative humidity of 55 ± 5%), were used. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups comprising of 8 mice. Each mouse was used only once and all tests were performed between 08.00 and 15.00 hours. Procedures involving animals and their care were conducted in accordance with current European Community and Polish legislation on animal experimentation. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. The experimental protocols and procedures described in this manuscript were approved by the Second Local Ethics Committee at the University of Life Sciences in Lublin (License no. 85/2009) and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Substances. The following substances were used in this study: arbutin, borneol, esculetin, esculin, ellagic acid, gallic acid, hesperidine, piperitol, piperonal, quercetin, thymoquinone and ursolic acid. All the tested substances were isolated from herbs and medicinal plants by Dr. M. Gleńsk and Dr. M. Włodarczyk from the Department of Pharmacognosy, Wroclaw Medical University (Wroclaw, Poland). All substances were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and were administered intraperitoneally (i.p.) as a single injection, in a volume of 5 ml/kg body wt. In the screening test, the studied compounds were administered i.p. at a constant dose of 300 mg/kg at four pretreatment times (i.e., 15, 30, 60 and 120 min) before the MES test.

Maximal electroshock-induced seizures. Electroconvulsions were produced by means of an alternating current (sine-wave, 0.2 s stimulus duration, 50 Hz, 25 mA, 500 V) delivered via ear-clip electrodes by a Rodent Shocker generator (Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The electrical system of the stimulator was self-adjustable so that changes in impedance did not result in alterations of current intensity (i.e., the system provides constant current stimulation). The criterion for the occurrence of seizure activity was the tonic hind limb extension (i.e., the hind limbs of animals outstretched 180° to the plane of the body axis). The protective activity of the various compounds in the screening test was determined as percentage of protection of animals against MES-induced tonic seizures. In the screening test, the studied com-

pounds were administered i.p. at a constant dose of 300 mg/kg at four pretreatment times (i.e., 15, 30, 60 and 120 min) before the MES test.

Statistics. Statistical analysis of data from the MES test was performed with Fisher's exact probability test. Differences among values were considered statistically significant if $P<0.05$. Statistical test was performed using commercially available GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Effects of arbutin, borneol, esculetin, esculin, ellagic acid, gallic acid, hesperidine, piperitol, piperonal, quercetin, thymoquinone and ursolic acid against maximal electroshock-induced seizures in mice in the screening test

None of the investigated substances, except for borneol, administered i.p. at a constant dose of 300 mg/kg produced a potent anticonvulsant effect against MES-induced tonic seizures in mice at the respective pretreatment times (Table 1). In case of borneol, the compound protected 6 out of 8 mice (a 75% of animals tested) when administered i.p. in a dose of 300 mg/kg at 15 min. before the MES test (Table 1). Systemic administration of borneol at 30 min. prior to the MES test resulted in a 37.5% protection (3 out of 8 mice) against MES-induced tonic seizures (Table 1). Prolongation of the pretreatment time to 60 min. before the MES test resulted in reduction of the anticonvulsant action of borneol, because only one mouse out of 8 mice (a 12.5% of animals tested) was protected against MES-induced tonic seizures (Table 1). In contrast, borneol administered i.p. in a dose of 300 mg/kg at 120 min. prior to the MES test produced no protection against tonic seizures in mice (Table 1). Statistical analysis of data with Fisher's exact probability test revealed that borneol administered i.p. at 15 min. before the MES test considerably protected the animals against MES-induced seizures ($P<0.01$; Table 1). In case of esculin, the substance administered i.p. at 30 min. before the MES-induced tonic seizures, protected one out of 8 mice (a 12.5% of animals tested). Similarly, thymoquinone when administered systemically (i.p.) at 15 and 30 min. before the MES test protected a 12.5% of animals tested (Table 1). In contrast, thymoquinone protected a 25% of animals tested (2 out of 8 mice) when administered i.p. at 60 min. after its systemic administration (Table 1).

DISCUSSION

The aim of this study was to perform the anticonvulsant screening test for 12 naturally occurring substances isolated from herbs and medicinal plants. As shown in Table 1, the tested compounds suspended in a 1% solution of Tween 80 in sterile saline in a constant dose of 300 mg/kg did not protect the animals against MES-induced tonic seizures, except for borneol, which protected maximally a 75% of animals tested (6 out of 8 mice) when administered i.p. at 15 min. after its systemic administration. Generally, it is widely accepted that the anticonvulsant effects are considered of pivotal importance if at least 50% of the animals tested are protected against seizures in the screening test.

Table 1. Time-course of anticonvulsant effects of the various naturally occurring compounds against maximal electroshock (MES)-induced seizures in mice

Pretreatment time (min.)	15	30	60	120
Substance	p/t (%)			
Vehicle	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)
Arbutin	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)
Borneol	6/8 (75)**	3/8 (37.5)	1/8 (12.5)	0/8 (0)
Esculetin	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)
Esculin	0/8 (0)	1/8 (12.5)	0/8 (0)	0/8 (0)
Ellagic acid	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)
Gallic acid	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)
Hesperidine	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)
Piperitol	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)
Piperonal	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)
Quercetin	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)
Thymoquinone	1/8 (12.5)	1/8 (12.5)	2/8 (25)	0/8 (0)
Ursolic acid	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)

Data are presented as number of animals protected against maximal electroshock (MES)-induced seizures out of 8 animals per group (and as % in parentheses). The MES test was performed at various pretreatment times (15, 30, 60 and 120 min.) after systemic (i.p.) administration of the investigated compounds in a constant dose of 300 mg/kg. p – number of animals protected against MES- induced seizures; t – number of animals tested per group. Statistical analysis of data was performed with Fisher's exact probability test. ** $P<0.01$ vs. the respective control group (vehicle-treated animals).

The anticonvulsant effects of borneol reduced along with the pretreatment time. It was documented that borneol administered systemically at a constant dose of 300 mg/kg at 15 min. before the MES test protected a 75% of animals tested and at 30 min. before the MES test, the compound exerted only a 37.5% protection against MES-induced tonic seizures. Subsequent prolongation of the pretreatment time to 60 min. resulted in a 12.5% protection of the tested animals in this seizure model. In contrast, borneol administered i.p. at 120 min before the MES test produced no anticonvulsant effects in experimental animals. Results indicate that the time to peak of anticonvulsant activity of borneol was established at 15 min. before the MES test. Results from this screening test revealed also that the remaining tested agents (i.e., arbutin, esculetin, esculin, ellagic acid, gallic acid, hesperidine, piperitol, piperonal, quercetin, thymoquinone and ursolic acid) have no clear-cut anticonvulsant activity in the mouse MES model, at the respective pretreatment times.

It is worthy of mentioning that the screening procedure applied in this study was almost identical to that accepted by National Institutes of Health (USA) and Antiepileptic Drug Development Program for searching for novel AEDs [23, 25]. There is no doubt that agents and compounds isolated from herbs and medicinal plants could

become a very important source to search for compounds possessing anticonvulsant properties in preclinical studies. It is widely accepted that herbs and plants contain a series of compounds that could be clinically favorable, especially, in epileptic patients.

Previously, we have documented that imperatorin, osthole and xanthotoxin (three naturally occurring coumarins isolated from various medicinal plants) exerted the anticonvulsant action in the mouse MES model [11, 14]. In contrast, bergapten and oxypeucedanin (two other naturally occurring coumarins) produced no or negligible anticonvulsant action against MES-induced tonic seizures in mice [11]. It is worthy of mentioning that the anticonvulsant potency of imperatorin, osthole and xanthotoxin has been evaluated in the screening test, identical to that applied in this study. Bearing in mind the fact that the screening procedure used in our previous studies was sensitive enough to detect the anticonvulsant action of xanthotoxin, osthole and imperatorin, we attempted to perform the screening test so as to detect the anticonvulsant action of substances isolated from various herbs and medicinal plants. There is no doubt that herbs and plants are the perfect source of active agents that would be used in patients with epilepsy. On the other hand, pharmaceutical companies and industries are able to create novel compounds more effective than their natural counterparts due to selective and rational modification of chemical structure of the investigated compounds. Of note, in our study we tested various compounds belonging to several diverse chemical families, including, coumarins, glycosylated hydroquinones, flavonoids, monoterpenes and triterpenes.

In this study we tested the agents only in the MES-induced tonic seizures in mice. However, as documented by National Institutes of Health (USA) and Antiepileptic Drug Development Program, the compounds should be tested in other experimental models of epilepsy, especially in the pentylenetetrazole-induced clonic seizure and 6 Hz (psychomotor) seizure tests in mice, in order not to omit any effective compounds [23, 25]. It should be mentioned that levetiracetam (a second-generation AED) is ineffective in the mouse MES model and pentylenetetrazole-induced clonic seizure test, however, the drug protected the animals against 6Hz induced psychomotor (limbic) seizures and displays strong anticonvulsant activity in this seizure model [25]. The example of levetiracetam prompted us to investigate the naturally occurring substances in the MES model as a first screening test. It is likely that agents ineffective in the MES test could suppress seizures in the pentylenetetrazole- or 6 Hz-induced seizure models in mice. However, more advanced experimental studies are required to confirm this hypothesis.

Generally, all the tested compounds administered systemically (i.p.) at a constant dose of 300 mg/kg produce no or negligible adverse effects in mice. No signs of ataxia or motor coordination impairment were observed in this study in animals receiving arbutin, borneol, esculetin, esculin, ellagic acid, gallic acid, hesperidine, piperitol, piperonal, quercetin, thymoquinone and ursolic acid (results not shown).

Summing up, the preclinical screening test of the various compounds isolated from herbs and medicinal plants provides evidence that borneol possesses the anti-seizure activity, while arbutin, esculetin, esculin, ellagic acid, gallic acid, hesperidine, piperitol, piperonal, quercetin, thymoquinone and ursolic acid are devoid of the anticonvulsant properties in the mouse MES model. It seems that only borneol is worthy of consideration as a potentially favorable therapeutic agent in epileptology, especially, if the results from this study could be extrapolated to the clinical settings.

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REFERENCES

1. Abelson P.H.: Medicine from plants. *Science*, 247, 513, 1990.
2. Blanco M.M. et al.: Neurobehavioral effect of essential oil of Cymbopogon citratus in mice. *Phytomedicine*, 16, 265, 2009.
3. Brodie M.J., Schachter S.C.: *Fast Facts. Epilepsy*, second ed., Health Press, Oxford 2001.
4. Heinrich M., Gibbons S.: Ethnopharmacology in drug discovery: an analysis of its role and potential contribution. *J. Pharm. Pharmacol*., 53, 425, 2001.
5. Heinrich M.: Ethnobotany and its role in drug development. *Phytother. Res.*, 14, 479, 2000.
6. Heinrich M.: Ethnobotany and natural products: the search for new molecules, new treatments of old diseases or a better understanding of indigenous cultures? *Curr. Top. Med. Chem.*, 3, 141, 2003.
7. Kwan P., Brodie M.J.: Early identification of refractory epilepsy. *N. Engl. J. Med.*, 342, 314, 2000.
8. Kwan P., Brodie M.J.: Epilepsy after the first drug fails: substitution or addition? *Seizure*, 9, 464, 2000.
9. Liao W.P. et al.: Study of antiepileptic effect of extracts from Acorus tatarinowii Schott. *Epilepsia*, 46 (Suppl 1), 21, 2005.
10. Löscher W., Fassbender C.P., Nolting B.: The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. II. Maximal electroshock seizure models. *Epilepsy Res*., 8, 79, 1991.
11. Łuszczki J.J. et al.: Anticonvulsant effects of four linear furanocoumarins, bergapten, imperatorin, oxypeucedanin, and xanthotoxin, in the mouse maximal electroshock-induced seizure model: a comparative study. *Pharmacol. Rep.*, 62, 1231, 2010.
12. Łuszczki J.J., Głowniak K., Czuczwar S.J.: Imperatorin enhances the protective activity of conventional antiepileptic drugs against maximal electroshock-induced seizures in mice. *Eur. J. Pharmacol*., 574, 133, 2007.
13. Łuszczki J.J., Głowniak K., Czuczwar S.J.: Time-course and dose-response relationships of imperatorin in the mouse maximal electroshock seizure threshold model. *Neurosci. Res*., 59, 18, 2007.

14. Łuszczki J.J. et al.: Anticonvulsant and acute neurotoxic effects of imperatorin, osthole and valproate in the maximal electroshock seizure and chimney tests in mice: a comparative study. *Epilepsy Res.*, 85, 293, 2009.
15. Łuszczki J.J. et al.: Influence of imperatorin on the anticonvulsant activity and acute adverse-effect profile of lamotrigine in maximal electroshock-induced seizures and chimney test in mice. *Pharmacol. Rep*., 60, 566, 2008.
16. Mandegary A. et al.: Anticonvulsant activity of the essential oil and methanolic extract of Bunium persicum (Boiss). *B. Fedtsch. J. Ethnopharmacol.*, 140, 447, 2012.
17. Mandegary A. et al.: Anticonvulsant activity and toxicity of essential oil and methanolic extract of Zhumeria majdae Rech, a unique Iranian plant in mice. *Neurochem. Res.*, 37, 2725, 2012.
18. Pourgholami M.H. et al.: Evaluation of the anticonvulsant activity of the essential oil of Eugenia caryophyllata in male mice. *J. Ethnopharmacol*., 64, 167, 1999.
19. Pourgholami M.H. et al.: The fruit essential oil of Pimpinella anisum exerts anticonvulsant effects in mice. *J. Ethnopharmacol*., 66, 211, 1999.
20. Raza M. et al.: Anticonvulsant activities of the FS-1 subfraction isolated from roots of Delphinium denudatum. *Phytother. Res.*, 15, 426, 2001.
21. Sayyah M., Nadjafnia L., Kamalinejad M.: Anticonvulsant activity and chemical composition of Artemisia dracunculus L. essential oil. *J. Ethnopharmacol*., 94, 283, 2004.
22. Sayyah M., Valizadeh J., Kamalinejad M.: Anticonvulsant activity of the leaf essential oil of Laurus nobilis against pentylenetetrazole- and maximal electroshock-induced seizures. *Phytomedicine*, 9, 212, 2002.
23. Stables J.P., Kupferberg H.J. *Chapter 16 - The NIH Anticonvulsant Drug Development (ADD) Program: preclinical anticonvulsant screening project.* In: *Molecular and cellular targets for anti-epileptic drugs.* Avanzini G., Regesta G., Tanganelli P., Avoli M. (eds). John Libbey, London 1997; p. 191.
24. Wahab A. et al.: Anticonvulsant activities of nutmeg oil of Myristica fragrans. *Phytother. Res*., 23, 153, 2009.
25. White H.S. et al.: *Discovery and preclinical development of antiepileptic drugs.* In: *Antiepileptic drugs. 5th edn.* Levy R.H., Mattson R.H., Meldrum B.S., Perucca E. (eds). Lippincott Williams & Wilkins, Philadelphia 2002; p. 36.