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Effect of Vanillin on Electrical and Chemical Induced Seizures in Rodents

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ABSTRACT

Vanilla planifolia grown for its attractive aroma has rich medicinal value as evidenced by its antimutagenic, antinvasive, anti-metastatic, antinociceptive property and protection against amygdala-kindled seizures. Lack of scientific data authentifying its antiepileptic potential prompted us to evaluate its antiepileptic activity in chemically and electrically induced seizures. Swiss albino mice and Wistar albino rats of either sex (n=6) were induced with seizures using Pentylenetetrazole (PTZ) 60mg/kg i.p. and Maximal electro shock (MES) (50mA for 0.2 seconds) respectively. Sodium valproate 100mg/kg and Phenytoin 25mg/kg served as controls for the respective groups. Vanillin was administered at 100, 200 and 500mg/kg per oral one hour prior to induction of convulsions. The parameters studied include: Onset and duration of tonic flexion and extension, onset of clonic seizure, time for recovery/ death (MES model); Onset of myoclonic jerks and number of episodes (PTZ model). Data were analyzed by one-way ANOVA followed by Dunnet's test. $P < 0.05$ was considered as statistically significant. In the MES model, vanillin showed a dose dependent significant decrease in the onset and duration of extension when compared to Phenytoin ($P< 0.01$). A similar decrease in the onset and duration of flexion was also noted at 100 and 200mg/kg but this was not significant at 500mg/kg dose. In the PTZ induced seizure model, acute administration of vanillin increased latency of onset and decreased the duration of seizures in treated animals as compared to the control. To conclude, the results suggest that vanillin has anticonvulsive property.

Keywords: Vanillin, maximal electroshock seizures, pentylenetetrazole, anticonvulsant

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INTRODUCTION

Epilepsy is a common and chronic neurological disorder characterized by apparently unprovoked, recurrent paroxysmal events or seizures that are associated with a sudden alteration in motor activity and behaviour with or without alteration in conscious awareness[1]. A seizureis a paroxysmal event due to abnormal, excessive, hyper synchronous discharges from an aggregate of central nervous system (CNS) neurons. Depending on the distribution of discharges, this abnormal CNS activity can have various manifestations, ranging from dramatic convulsive activity to experiential phenomena not readily discernible by an observer [2].

Gamma-Aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the cerebral cortex, maintains the inhibitory tone that counterbalances neuronal excitation; any imbalance in GABA leads to epilepsy[3]. Using the definition of epilepsy as two or more unprovoked seizures, the incidence of epilepsy is 0.3–0.5% in different populations throughout the world[2]. Approximately one-third of patients with epilepsy do not respond to treatment with a single antiepileptic drug, and it becomes necessary to try a combination of drugs to control seizures. It has been estimated that 20–30% of patients with epilepsy are resistant to medical therapy despite efforts[4]. Although many drugs are available in the market, much effort is being devoted to novel approaches which have a broad antiepileptic spectrum with least adverse effect profile, as these drugs have to be given for a very long duration. Hence the search for newer and better antiepileptic drugs continues to exist.

Vanilla (*Vanilla planifolia*), a monocotyledonous orchid, native of Central America, is grown for the attractive aroma produced by its fruit[5]. The increase in demand as well as its cost has prompted vanilla users to utilize synthetic vanilla instead of natural vanilla. In fact, 97% of vanilla used as a flavour and fragrance is synthetic. Synthetic vanilla contains only one organic component - vanillin- the flavour and fragrance that we most associate with vanilla. Natural vanilla extract is a mixture of several hundred different compounds in addition to vanillin. Vanillin is one of the primary chemical components of the extract of the vanilla bean (*Vanilla planifolia*). It is a pleasant aromatic compound that occurs naturally in vanilla beans. It is a fine, white to slightly yellow crystal, usually needle-like, having an odour and taste suggestive of vanilla. Synthetic vanillin is used as a flavoring agent in foods, beverages, and pharmaceuticals[6]. Various previous studies on vanillin have demonstrated that it has antimutagenic[7], antinvasive[8,9]and metastatic suppression potential[8,9] by inhibiting enzymatic activity of matrix metaloproteinase-9[9]. It has also shown to have antinociceptive property in acetic acid induced

visceral inflammation pain model[10]. Ge Q et al have demonstrated its protective role in fully amygdala-kindled seizures[11]. However, literature search revealed no further progress or additional studies regarding its antiepileptic potential. Hence the present study was designed to evaluate the antiepileptic activity of vanillin in chemically and electrically induced seizure models in rodents.

MATERIALS AND METHODS:

Animals:

Healthy adult albino mice (Swiss Strain) and Wistar albino rats of either sex weighing 20-30 gram and 150-200 gram respectively, inbred in our own central animal house (KMC, Mangalore) were used for the study. Rodents were housed in clean polypropylene cages, with dust free rice husk as a bedding material; six mice in each cage and three rats per cage; under controlled laboratory conditions (Temperature: 25° ± 2°C, humidity (60% ± 10%) and 12 h light/dark cycle as per CPCSEA guidelines). The experimental animals were fed with standard chow containing fat 4.15%, protein 22.15%, carbohydrates 4% (supplied by Amruth laboratory animal feed manufactured by Pranav Agro industries ltd., Sangli) and water *ad libitum*. The rodents were allowed to acclimatize to these conditions for one week prior to the commencement of the study. Experiments were performed during the light phase of the cycle (10:00-17:00).

Study Drug:

Vanillin [IUPAC name 4-hydroxy-3-methoxybenzaldehyde, chemical formula $(CH_3O)(OH)C_6H_3CHO$, molecular weight of 152.15] was obtained from Hi Media laboratories. The standard drugs Phenytoin (Sun Pharmaceuticals) was administered at a dose of 25mg/kg[12]by the intraperitoneal route (i.p.) and Sodium valproate (Sun Pharmaceuticals) was administered at the dose of 100mg/kg[13] by the oral route.

Study procedure

a) Pentylenetetrazole (PTZ) induced convulsions [1] :

The albino mice were selected two weeks prior to conducting the experiment by injecting PTZ (dissolved in normal saline), a standard convulsing agent at a dose of 60mg/kg i.p.[1]Mice which showed clonic convulsions within 30 minutes during preliminary examination were chosen for further study. Mice were randomly assigned to five groups of six mice each. The feeding and treatment schedule were as follows.

Group 1: Distilled water (Normal control) (10 ml/kg)

Group 2: Sodium valproate 100mg/kg per oral.

Group 3: Vanillin (dissolved in distilled water) 10mg/kg[10] per oral.

Group 4: Vanillin (dissolved in distilled water) 100mg/kg[8] per oral.

Group 5: Vanillin (dissolved in distilled water) 200mg/kg per oral

All drugs were prepared fresh and given 30 minutes prior to PTZ administration. Following observations were monitored.

- Onset of myoclonic jerks and number of episodes
- Onset and number of episodes of clonic seizure with loss of righting reflex

a) The maximal electroshock (MES) model[15]

The animals were chosen by preliminary screening. Rats which showedextension of hind limb were included in the study.The rats were randomly assigned into five groups of six animals each.

Group 1: Distilled water (Normal control)

Group 2: Phenytoin i.p. 25mg/kg[16]

Group 3: Vanillin (dissolved in distilled water) 10mg/kg[10] per oral.

Group 4: Vanillin (dissolved in distilled water) 100mg/kg[8] per oral.

Group 5: Vanillin (dissolved in distilled water) 200mg/kg per oral

The drugs were given one hour prior to induction of convulsions. Seizures were induced in the experimental animals by giving an electrical stimulus of 50mA for 0.2 seconds using electro-convulsiometer (MAC Laboratory equipments) and the duration of tonic hind limb extension was noted. The other parameters that were recorded include

- Onset and duration of tonic flexion and extension
- Onset of clonic seizure
- Time required as for recovery or death

Statistical analysis:

Statistical analysis were carried out using the software package SPSS (Version 17.0). The data was analyzed by one-way ANOVA with drug treatment as the independent factor. Post-hoc comparisons were performed by applying Dunnet's multiple comparison test. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION:

The MES profile consisted of two phases: [17]

a) The ictal phase-comprising tonic flexion of fore and hind limbs; Hind limb tonic extension (HLTE) and terminal clonus and

b) The post ictal depression (PID) which was the time from end of HLTE to the time when the animal walked away after regaining forelimb righting reflex.

In the present study, abolition of HLTE was taken as the index of anticonvulsant activity.

As depicted in table 1, the standard drug phenytoin abolished the HLTE completely when compared to the control group. The test drug vanillin though decreased the duration of HLTE (compared to the control) at all doses statistical significance was observed only at 100mg/kg and 200mg/kg doses, the effect being more at the higher dose. However, this dose dependent effect was not reflected at 500mg/kg dose which showed no statistical significance when compared to the lower doses.

Table 1 shows the effect of the test drug vanillin in MES induced convulsions in mice

Group	Onset of tonic flexion (sec)	Duration of tonic flexion (sec)	Onset of tonic extension (sec)	Duration of tonic extension (sec)	Onset of GTCS (sec)	Recovery(min)
Control	1.77±0.30	5.37±0.46	7.14±0.64	10.62±0.69	18.19±0.99	2.35±0.06
Standard drug-phenytoin	3.82±0.45*	1.96±0.31*	0.00±0.00*	0.00±0.00*	15.22±9.77	1.20±0.05*
Vanillin 100mg/kg	2.04±0.25#	4.29±0.43#	6.33±0.26#	8.06±0.37*#	17.96±1.00#	1.90±0.14*#
Vanillin 200mg/kg	2.34±0.20#	3.27±0.26*#	5.61±0.25#	4.93±0.15*#	6.24±6.23*#	1.76±0.13*#
Vanillin 500mg/kg	2.86±0.11	2.96±0.10	5.82±0.15#	5.34±0.22#	11.40±5.23	1.37±0.06

- *Significant when compared to control $p<0.05$
- # significant when compared to standard drug, phenytoin $p<0.05$
- Values are expressed as MEAN±SEM

Compared to the standard Phenytoin, vanillin also exhibited statistically significant decrease in the onset and duration of tonic flexion and terminal clonus at 100mg/kg and 200mg/kg doses. The decrease observed at 500mg/kg was not statistically significant. The recovery time was significantly decreased in all the vanillin treated groups as well as the phenytoin group when compared with the control.

As depicted in table 2, Sodium valproate increased the time of onset of myoclonic jerks as well as clonic seizures and significantly decreased the no. of episodes respectively. The test drug, vanillin significantly increased both the onset as well as decreased the number of episodes of both myoclonic jerks and clonic seizures at all the doses used when compared to the control. ($P<0.01$) But when compared to the positive control, sodium valproate statistical significance was obtained only in terms of the decrease in number of myoclonic jerks and clonic seizures.

Table 2 shows the effect of the test drug vanillin in PTZ induced convulsions in mice.

Groups	Onset of myoclonic	No of episodes of myoclonic	Onset of clonic	No of episodes of clonic seizures

	jerks(sec)	jerks	seizures(min)	
Control	12.70±2.02	25.50±1.80	1.23±0.09	2.67±0.33
Standard drug sodium valproate	75.15±25.36	1.17±0.401*	5.561±3.63	0.50±0.22*
Vanillin 100mg/kg	34.76±3.18*	17.33±0.56*#	1.90±0.14*	1.50±0.22*#
Vanillin 200mg/kg	54.62±3.30*	11.83±0.83*#	4.00±0.34*	1.33±0.21*#
Vanillin 500mg/kg	64.35±5.56*	9.50±0.62*#	5.25±0.43*	1.17±0.17*#

- *Significant when compared to control $p<0.05$
- # significant when compared to standard drug, Sodium valproate $p<0.05$
- Values are expressed as MEAN±SEM

Vanillin, the major constituent of vanilla beans, is one of the most important aromatic flavor compounds used in foods, beverages, perfumes, and pharmaceuticals and is produced on a scale of more than 10 thousand tons per year by the industry through chemical synthesis. Previous studies showing the effect of vanillin in amygdala-kindled seizures in rats[11]suggest evidence for the antiepileptic potential for vanillin. However literature search revealed no studies conducted on chemical and electrically induced seizures. Hence this study was conducted to evaluate the antiepileptic potential of vanillin in MES and PTZ induced convulsions in Wistar albino rats.

In the present study, Vanillin on acute administration reduced the tonic hind limb extensor phase in MES induced seizures in experimental animals in a dose dependent manner. Also, when the convulsive challenge was made with PTZ, acute administration of vanillin increased the latency of onset and decreased the duration of seizures in the treated animals as compared to the control.

GABA is an inhibitory neurotransmitter while glutamic acid is an excitatory neurotransmitter in the brain. Inhibition of GABA and enhancement of glutamate activity have been shown to be underlying factors in epilepsy[18,19].

The maximal electroshock test is the most widely used animal model in antiepileptic drug discovery, because seizure induction is simple and the predictive value for detecting clinically effective antiepileptic is high[20,21]. It has often been stated that antiepileptic drugs that block MES-induced tonic extension act by blocking seizure spread. Moreover, MES-induced tonic extension can be prevented either by drugs that inhibit voltage-dependent Na+ channels, such as phenytoin, valproate, felbamate and lamotrigine or by drugs that block glutamatergic excitation mediated by the N-methyl-Daspartate (NMDA) receptor, such as felbamate[22].The maximal electroshock test identifies agents with activity against generalized tonic clonic seizures using clinically established antiepileptic drugs[20]. The pharmacology of acute maximal electroshock does not differfrom the pharmacology of generalized tonic-clonic seizures in genetic models with chronic epilepsy[21]. Since inhibition of the MES test predicts activity against generalized tonic-

clonic and cortical focal seizures so activity against MES induced seizures suggests that vanillin is useful in suppressing generalized tonic-clonic seizures by regulating GABA mediated synaptic inhibition through an action at distinct sites of this synapsis. It could also be proposed that vanillin could also have blocked the seizure spread by inhibiting Na^+ channels and glutamatergic excitation through NMDA receptor.

On the other hand, the pentelenetetrazole test represents a valid model for human generalized myoclonic and also absence seizures. Hence the present data suggests that vanillin could be useful in absence and myoclonic seizures. In general, compounds with anticonvulsant activity in the petitmal epilepsy are effective in pentelenetetrazole-induced seizure model[23]. Drugs that reduce T-type Ca2+ currents, such as ethosuximide can prevent seizures induced by PTZ[24]. The clonic seizure in PTZ model could be due to decreased seizure threshold[25]. According to be Sarro et al, pentelenetetrazole may be exerting its convulsive effect by inhibiting the activity of gamma amino butyric acid (GABA) at GABA A receptors (the major inhibitory neurotransmitter which is implicated in epilepsy[26].The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion respectively[27,28,29]. Since vanillin delayed the occurrence of pentelenetetrazole-induced convulsion, it is probable that it may be interfering with GABA aminergic mechanism(s) and Ca^{2+} channels to exert its anticonvulsant effect.

To conclude, the results of the present studies demonstrated antiepileptic potential of vanillin against MES and PTZ induced seizures. Further studies are needed to ascertain its clinical effectiveness, safety profile in chronic dose studies and elucidate its exact mechanism of action.

REFERENCES

1. Brahmane RI, Pathak SS, Salwe KJ, Wanmali VV. Role of cinnarizine and nifedipine on anticonvulsant effect of sodium valproate and carbamazepine in maximal electroshock and pentylenetetrazole model of seizures in mice. J Pharmacol Pharmacother 2010;1(2):78-81.
2. Daniel H. Lowenstein. Seizures and epilepsy. In: Anthony S. Fauci, Dennis L. Kasper, Dan L. Longo, Eugene Braunwald, Stephen L. Hauser, J. Larry Jameson , et al, editors. Harrison's principles of internal medicine. 17th ed. New York: McGraw Hill, Health Professions Division; 2008.
3. David M. Treiman. GABAergic mechanisms in epilepsy. Epilepsia 2001;42 (3): 8-12.

4. McNamara JO. Pharmacotherapy of the epilepsies. In: Burton LL, Lazo JS, Parker KS, editors. Goodman & Gillman's the pharmacological basis of therapeutics 11th ed. New York, McGraw-Hill, Medical publishing division; 2006.
5. Brillouet JM, Escoute J, Odoux E, Verdeil JL. Localization of β-D_Glucosidase activity and glucovanillin in vanilla Bean (*Vanilla planifolia* Andrews). Annals of Botany 2003; 92:437-44.
6. Sweetman SC. Martindale The Complete Drug Reference. 36th ed. London: pharmaceutical Press; 2009.
7. Kinga AA, Leszczynska J, Murea K, Shaughnessy DT, Umbach DM, Ward WO, et al. Antimutagenicity of cinnamaldehyde and vanillin in human cells: global gene expression and possible role of DNA damage and repair. Mutat Res 2007; 616(1-2): 60–9.
8. Aozuka Y, Choo M, Kawasaki N, Lirdprapamongkol K, Saitoh Y, Sakurai H, et al. Vanillin suppresses in vitro invasion and in vivo metastasis of mouse breast cancer cells. Eur J Pharm Sci 2005; 25:57-65.
9. Hsiang C, Liang J, Wu S, Lo H, Ho T. Vanillin inhibits matrix metalloproteinase-9 expression through down-regulation of nuclear factor-B signalling pathway in human hepatocellular carcinoma cells. Mol Pharmacol 2009; 75:151–7.
10. Choi S, , Kwon M, Lee J, Park S, Seo Y, Sim Y et al. Antinociceptive profiles and mechanisms of orally administered vanillin in the mice. Arch Pharm Res 2009; 32(11): 1643-9.
11. Ge Q, Jin H, Liu GQ, Jin XN, Wu HQ, Xie L. The effect of vanillin on the fully amygdala-kindled seizures in the rat. Yao XueXueBao. 1989; 24(7):482-6.
12. Bothara SB, Golwala DK, Mani M, Patel LD, Patel P, Vaidya SK. Anticonvulsant activity *of Abutilone indicum* leaf. Int J Pharm and Pharma Sci 2010; 2(1): 66-71.
13. Rehni AK, Singh N. Reversal of pentylenetetrazole-induced seizure activity in mice by nickel chloride. Indian J Pharmacol 2009; 41(1):15-18.
14. Chakrabarti A, Hota D, Sahai AK, Shitak R. Anti-seizure efficacy of nimodipine in pentylenetetrazole and kainic acid combined seizure models in mice. Indian J Physiol Pharmacol 2006; 50 (3) : 265–272.
15. Bhalla S,Khanna N, Sharma KK, Verma V. Modulatory effects of nifedipine and nimodipine in experimental convulsions. Indian J Pharmacol 2000; 32 :347-352.

16. Rajkapoor B, Senthil Kumar KK. Effect of *Oxalis corniculata* L. extracts on biogenic amines concentrations in rat brain after induction of seizure. International Journal of Phytopharmacology 2010; 1(2):87-91.
17. David J, Joseph S, Joseph T. Additive anticonvulsant effect of flunarizine and sodium valproate on electroshock and chemoshock induced seizures in mice. Indian J Physiol Pharmacol 1998;42: 383-8.
18. Westmoreland BF, Benarroch EE, Dube JR, Regan TJ, Sandok BA. Medicinal neurosciences. Rochester: Margo Foundation, 1994, pp.307-312.
19. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. Churchill Livingstone, Edinburgh, 2003, 5thed, pp. 557–560.
20. White HS. Clinical significance of animal seizure models and mechanism of action, studies of potential antiepileptic drugs. Epilepsia 1995; 38 Suppl: S9-17
21. Loscher W. Animal models of epilepsy for the development of antileptogenic and disease modifying drugs: A comparison of pharmacology of kindling and poststatus epilepticus models of temporal lobe epilepsy. Epilepsy Res. 1999; 50: 105-23.
22. Mohammed Sayyah, Ali Mandgray, Mohammad Kamalinejad. J Ethnopharmacol 2002; 82: 105-109.
23. W. Loscher, D. Schmidt. Which animal's models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical consideration. Epilepsy Res 1988; 2: 145-18.
24. Karunakar Hegde, Shalin P Thakker, Arun B Joshi, Shastry CS, Chandrashekhar KS. Trop J Pharm Res 2009; 8(2): 117-125.
25. Olufunmilayo O Adeyemi, Omoniyi K Yemitan, Olayemi O Adebiyi. J Ethnopharmacol 2007; 113: 111-114.
26. De Sarro A, Cecchetti V, Fravolini V, Naccari F, Tabarrini O,De Sarro G. Effects of novel β-desfluroquinolones and classic quinolones on pentelenetetrazole-induced seizures in mice. Antimicrob Agents Chemother. 1999; 43: 1729-36.
27. Westmoreland BF, Benarroch EE, Dube JR, Regan TJ, Sandok BA. Medical neurosciences. Mayo Foundation Rochester 1994, pp 307-12.
28. Gale MK. GABA and epilepsy basic concepts from preclinical research. Epilepsia 1992; 33 Suppl: S3-12.
29. Meldrum BS. GABA agonists as antiepileptic agents. Advan Biochem Psycopharmacol. 1981; 26: 207-17.