Official Publication of the Faculty of Basic Medical Sciences University of Ibadan, Nigeria

Arch. Bas. App. Med. 4 (2016) 121 – 124
www.archivesbamui.com

ARCHIVES OF BASIC & APPLIED MEDICINE

Research Article

Anticonvulsant Effects Virgin Coconut-oil, Sodium-valproate and Phenobarbital in Wistar-Rats

Yusuf A, Alhassan A.W, Saleh M.I.A, Adamu B.Y, Olaide L, Jamila Z, Aisha Y

[1]*Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria, West Africa*

Abstract

A comparative study of the anticonvulsant efficacies of Virgin Coconut Oil (VCO) and known Anti-Epileptic Drugs (AEDs) - Phenobarbital and Sodium Valproate- were undertaken using the method maximal electroshock (MES). The modulatory effect of VCO on regular AEDs was evaluated. Thirty male Wistar rats (180-220 grams) were randomly allocated into six groups of five rats each. Groups IV, V and VI received 10 ml/kg/day/6wks/oral-route of VCO while Groups I, II and III were similarly handled but received 5 ml/kg 0.9% normal saline. After 6 weeks, Groups II and V received 50 mg/kg/i.p Phenobarbital while Groups III and VI received 400 mg/kg/i.p Sodium Valproate. Groups IV and I served as the positive and negative control groups respectively. Thirty (30) minutes after treatment with AEDs, by a slid-up-type voltage regulator: P.I-240V, 50/60Hz; P.O-0-240V, 5AMP, 80volts/60Hz/sec electroshock was delivered through ear-clip electrodes for induction of seizure and the duration of the seizure was noted as the time from seizure onset to when rat shows reflex withdrawal of its limb when extended. Data were analyzed by Student's t-test. The results showed that seizure duration was significantly ($P < 0.05$) reduced by VCO, Sodium Valproate and Phenobarbital in electroshock induced seizures. The anticonvulsant efficacy of VCO was lower than that of Sodium valproate but comparable with that of Phenobarbital. The reduction effect of Sodium Valproate and Phenobarbital were potentiated when administered together with VCO. Combined-therapy with VCO may be considered for the treatment of epileptic conditions resistant to treatments with Sodium Valproate and Phenobarbital.

Keywords: Virgin coconut-oil (VCO), maximum electroconvulsive shock, Phenobarbital and Sodium Valproate

INTRODUCTION

Virgin Coconut Oil (VCO) is a plant extract from the kernel of matured coconut palm Cocos nucifera. It is composed predominantly of a special group of saturated fatty acids known as medium chain triglycerides (MCT). The MCT are made up of lauric acid, capric acid, caprylic acid, myristic acid and palmitic acid (Van de Kamer, 1958). VCO possess immense medicinal values with several applications in complementary medicine (Cross, 2010; Kossoff, 2013). In Nigerian, it is used in traditional medicine for the management of epileptic seizures in children and its efficacy is widely acclaimed amongst the Yoruba communities in the South-west of Nigeria. Moreover, VCO has repeatedly appeared in several internet and newspaper anecdotes as providing efficacious protection against epileptic seizures intractable to available antiepileptic drugs (AEDs) (Vining and Freeman, 1998; Kossoff et al., 2003; McDougall, 2006; Neal et al., 2009).

In spite of the currently available improved antiepileptic drugs (AEDs), almost one-third of epileptic patients continue to present seizures that appear to be refractory to all pharmacological schemes (Bialer, 2006; Perucca et al., 2007). Moreover, AEDs are associated with serious side-effects including teratogenicity and adverse-effect on cognitive functions (Samren et al., 1997; Hermann et al., 2010). Therefore, in the search for treatments that are more effective and safer than the available AEDs, family caregivers are quick to adopt treatments with VCO without seeking the supervision of healthcare providers and in most cases, VCO is administered in combination with patients' AEDs prescription by family caregivers, yet, its modulatory effects on the efficacies of the AEDs cannot be disregarded.

When asked for recommendations on the use and efficacy of VCO, physicians have inadequate peer-reviewed research information about the benefits and efficacy of VCO to make research-based care recommendations. Therefore, a comparative study of the anticonvulsant efficacies of VCO and regular AEDs, and the modulatory effect of VCO on the efficacies of regular AEDs are required to inform decision making on the use of VCO, especially in the clinical setting. To this end, the anticonvulsant efficacies of VCO, Phenobarbital and Sodium Valproate, and the modulatory effect of VCO were determined by the maximal electroshock (MES) method.

MATERIALS AND METHODS

Preparation of virgin coconut-oil

Virgin coconut-oil was prepared according to *Nevin and Rajamohan*, (2004). The solid endosperm of mature coconut (i.e. *copra*) was obtained and crushed in an electric-blender into viscous slurry which was squeezed through cheese cloth to obtain coconut milk. The milk was refrigerated for 48 h. After 48 h, the milk was subjected to mild heating (50 °C) in a thermostat oven. Virgin oil was then filtered through cheesecloth and was used for the present study.

**Author for Correspondence:* +2348033629449 E-mail: *yusufbabs2003@yahoo.com*

Chemicals

Commercial grade Phenobarbital sodium and Sodium Valproate were purchased from the pharmaceutical store of Ahmadu Bello University Teaching Hospital (ABUTH) Zaria using a doctor's prescription note. Phenobarbital sodium injection was supplied in ampoules (200 mg/ml) and Sodium Valproate in tablet formulation (200 mg/tablet). Appropriate dilutions were made with deionized water prior to use. All other reagents used for the present study were of analytical grade.

Animals and treatments

Thirty (30) male Wistar rats weighing (180-220) grams, obtained from the animal facility of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, were randomly allocated into six (6) study groups of Five (5) rats per group. All rats were housed under controlled laboratory conditions of 12 hour light/dark cycles and temperature (25 ± 2 °C) with assess to feed (Vital Feeds, Jos) and water *ad libitum.* Groups I, II and III received 10 ml/kg/day/6wks/oral-route (o.r.) of VCO while Groups IV, V and VI were similarly handled but received 5 ml/kg 0.9% normal saline. After 6 weeks, Groups II and V received 50 mg/kg/i.p Phenobarbital while Groups III and VI received 400 mg/kg/o.r. Sodium Valproate. Groups IV and I served as the negative and positive control groups respectively. The procedures used were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Maximal electroshock-induced seizure

The electroshock methods according to *Swinyard* (1972), *Browning* (1992) and *Castel-Branco et al.* (2009) were modified and employed for the present study. Thirty (30) minutes after the treatments with the AEDs, by a slid-up-type voltage regulator: P.I-240V, 50/60Hz; P.O-0-240V, 5AMP, a single 80volts/60Hz/sec electroshock was delivered through ear-clip electrodes to each rat for induction of seizure characterized by tonic hind-limb extension and flexion, followed by clonus. Duration of the seizure was noted as the time from seizure-onset to when rat shows reflex withdrawal of its limb when extended by the investigator.

Statistical analysis

Data obtained were expressed as mean ± S.E.M. Student's *t*-test was used to determine level of significance of all results obtained. Results were regarded as significant at $p < 0.05$..

RESULTS

The effects of virgin coconut oil, phenobarbital and sodium valproate on seizure duration of electroshock-induced seizures are shown in Figure 1. The results indicate that seizure duration was significantly ($P < 0.05$) reduced by VCO, sodium valproate and phenobarbital and the anticonvulsant efficacy of VCO was lesser than that of sodium valproate but comparable with that of phenobarbital.

Figure 2 shows the effect of combined administration of virgin coconut oil with phenobarbital or with sodium valproate on seizure duration of electroshock induced seizures. The results indicated that the reduction effects of sodium valproate and phenobarbital were potentiated when administered in combination with VCO.

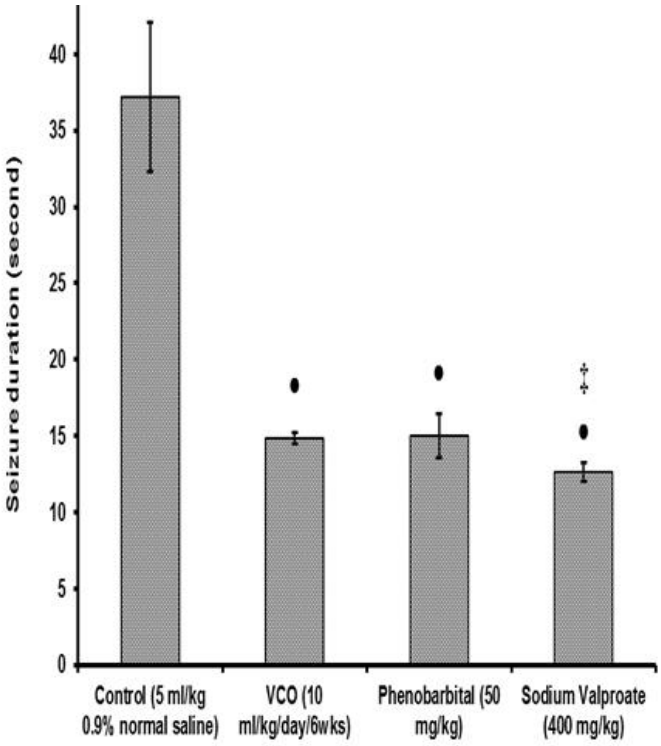

Figure 1

Effects of virgin coconut oil, phenobarbital and sodium valproate on seizure duration of electroshock induced seizures •$P < 0.05$ vs. control, ‡$P < 0.05$ vs. VCO.

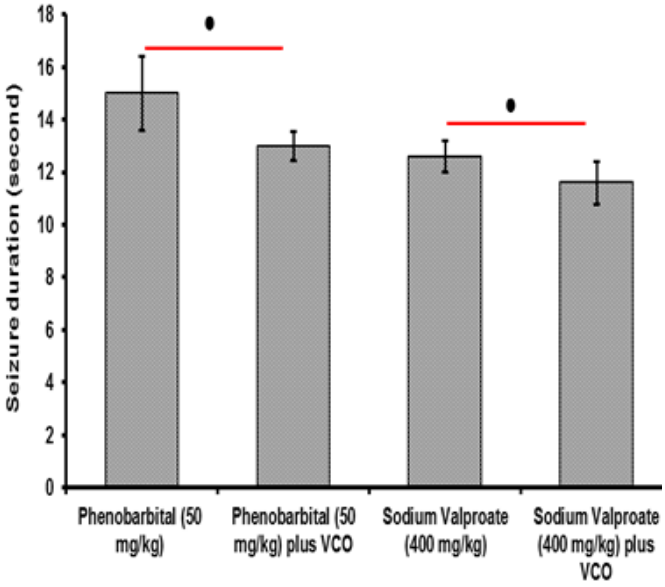

Figure 2

The effects of combined administration of virgin coconut oil with phenobarbital or with sodium valproate on seizure duration of electroshock induced seizures •$P < 0.05$.

DISCUSSION

The significance of research-based care recommendations on the anticonvulsant efficacy of VCO and its modulatory effect on regular AEDs by physicians cannot be disregarded, especially while counseling epileptic patients and family caregivers on the therapeutic benefits of VCO. The present study provides information on the anticonvulsant efficacies of VCO, Phenobarbital and Sodium Valproate, and the

modulatory effect of VCO as determined by the maximal electroshock (MES) method. Maximal electroshock (MES) is arguably a useful animal model of seizure; in particular, this model is often used to study the anticonvulsant efficacy of potential drug candidates (Swinyard, 1972; Browning, 1992; Castel-Branco *et al.,* 2009). MES-induced seizure shows tonic hind-limb extension and flexion, followed by clonus (Castel-Branco *et al.,* 2009). Seizure-duration is the critical variable in MES and a measure of the anticonvulsant efficacy of the drug being studied. Reduced seizure-duration is an indication of improved anticonvulsant efficacy (Swinyard, 1972; Browning, 1992; Castel-Branco *et al.,* 2009).

The results of the present study showed that VCO, sodium valproate and phenobarbital significantly ($P < 0.05$) reduced the seizure-duration of electroshock-induced seizure in Wistar rats but the reduction effect of VCO was significantly ($P < 0.05$) lesser than that of sodium valproate (Figure 1). These results put together is in discordance with the anecdotal reports that VCO provide best efficacy in seizure suppression compared with regular AEDs (Vining and Freeman, 1998; Kossoff *et al.,* 2003; McDougall, 2006; Neal *et al.,* 2009) and it suggest that sodium valproate is more efficacious than VCO but the efficacy of VCO is comparable with that of phenobarbital in electroshock-induced seizure in Wistar rats. Furthermore, the results on the modulatory effect of VCO on the efficacies of sodium valproate and phenobarbital showed that the reduction effects of sodium valproate and phenobarbital on the seizure-duration of electroshock-induced seizure were potentiated when administered in combination with VCO in Wistar rats (Figure 2). The results suggest that the combine administration of VCO with sodium valproate or with phenobarbital modulates the anticonvulsant effects of these regular AEDs by improving the seizure-suppressing efficacy of the drugs.

Sodium valproate is a weak blocker of sodium ion channels, inhibitor of GABA transaminase and a probable enhancer of GABA synthesis whereas, phenobarbital has a direct action on $GABA_A$ receptors, it prolongs the duration of chloride channel opening by binding to the barbiturate-binding site on the receptor. It also reduces sodium and potassium conductance and calcium influx and depresses glutamate excitability (Meldrum and Rogawski, 2007; Brodie *et al.,* 2011). It is not unlikely that VCO is acting via similar mechanisms of action to elicit its potentiating effect on the anticonvulsant action of sodium valproate or phenobarbital in the electroshock-induced seizure in Wistar rats. However, the anticonvulsant effect of VCO is often attributed to the seizure suppressing effect of the metabolites of its medium chain fatty acids (MCFAs) constitutes. MCFAs are metabolized by the liver via β-oxidation to produce ketone bodies, principally β-hydroxybutyrate (β-HB), acetone and acetoacetate (ACA). This results into elevated levels of circulating ketone bodies (ketosis), providing an alternative substrate to glucose for energy utilization by neurons and glia in the brain. Seizure protection in early clinical studies of dietary treatment of epilepsy has been attributed to ketosis, and the ketone bodies have been reported to possess anticonvulsant-property but the mechanisms of action the ketone bodies are not completely elucidated (Keith, 1933; Rho *et al.,* 2002; Freeman *et al.,* 2006; Bough and Rho, 2007; Hartman *et al.,* 2007).

In conclusion, the reduction effects of sodium valproate and phenobarbital on seizure-duration of electroshock-induced seizure were potentiated when administered together with VCO. Combined-therapy with VCO may be considered for the treatment of epileptic conditions resistant to treatments with sodium valproate and phenobarbital

REFERENCES

Bialer, M. (2006). New antiepileptic drugs that are second generation to existing antiepileptic drugs. Expert Opinion on Investigational Drugs, 15(6): 637-47.

Bough, K.J. and Rho, J.M. (2007). Anticonvulsant mechanisms of the ketogenic diet. Epilepsia, 48: 43-58.

Brodie, M.J., Covanis, A., Gil-Nagel, A., Lerche, H., Perucca, E., Sills, G.J. and White, H.S. (2011). Antiepileptic drug therapy: does mechanism of action matter? Epilepsy Behavior, 21(4): 331-41.

Browning, R. (1992). The electroshock model, neuronal network and antiepileptic drugs. In: Drugs for Control of Epilepsy: Actions on Neuronal Networks in Seizure Disorders, (Faingold, C.L. and Fromm, G.H. Eds.) CRC Press. Boca Raton, FL. Pp. 195-211.

Castel-Branco, M.M., Alves, G.L., Figueiredo, I.V., Falcão, A.C. and Caramona, M.M. (2009). The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. Methods and Findings in Experimental and Clinical Pharmacology, 31(2): 101-106.

Cross, J.H. (2010). Dietary therapies an old idea with a new lease of life. Seizure, 19(10):671-674.

Guide for the care and use of laboratory animals, DHEW Publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20892.

Hartman, A.L., Gasior, M., Vining, E.P. and Rogawski, M.A. (2007). The neuropharmacology of the ketogenic diet. Pediatric Neurology, 36(5):281-292.

Hermann, B., Meador, K.J., Gaillard, W.D. and Cramer, J.A. (2010). Cognition across the lifespan: Antiepileptic drugs, epilepsy, or both? Epilepsy and Behavior, 17: 1-5.

Keith, H. (1933). Factors influencing experimentally produced convulsions. Archives of Neurology and Psychiatry, 29: 148-154.

Kossoff, E.H. (2013). Nonpharmacological approaches: diet and neurostimulation. Handbook of Clinical Neurology, 111:803-808.

Kossoff, E.H., Krauss, G.L., McGrogan, J.R. and Freeman, J.M. (2003). Efficacy of the Atkins diet as therapy for intractable epilepsy. Neurology, 61: 1789-1791.

McDougall, J. (2006). The newest food-cure: coconut oil for health and vitality. The McDougall Newsletter, 5:5.

Meldrum, B.S. and Rogawski, M.A. (2007). Molecular targets for antiepileptic drug development. Neurotherapeutics, 4:18-61.

Neal, E.G., Chaffe, H., Schwartz, R.H., Lawson, M.S., Edwards, N., Fitzsimmons, G., Whitney, A. and Cross, J.H. (2009). A randomized trial of classical and medium-chain triglyceride ketogenic diets in the treatment of childhood epilepsy. Epilepsia, 50: 1109-1117.

Nevin, K.G. and Rajamohan, T. (2004). Beneficial effects of virgin coconut oil on lipid parameters and in-vitro LDL oxidation. Clinical Biochemistry, 37: 830-835.

Perucca, E., French, J., Bialer, M. (2007). Development of new antiepileptic drugs: Challenges, incentives, and recent advances. Lancet Neurology, 6(9): 793-804.

Rho, J.M., Anderson, G.D., Donevan, S.D. and White, H.S. (2002). Acetoacetate, Acetone, and Dibenzylamine (a Contaminant in L-(+)-beta-Hydroxybutyrate) Exhibit Direct Anticonvulsant Actions in Vivo. Epilepsia, 43(4): 358-361.

Samren, E.B., van Duijin, C.M. and Koch, S. (1997). Maternal use of antiepileptic drugs and the risk of major congenital maformations: A joint European prospective study of human teratogenesis associated with maternal epilepsy. Epilepsia, 38: 981-990.

Swinyard, E.A. (1972). Electrically-induced convulsions. In: Experimental Models of Epilepsy. A manual for the Laboratory Worker, (Purpura, D.P., Tower, D.B., Woodbury, D.M. and Walter, R.D. Eds.). Raven Press: New York. Pp. 433-458.

Van de Kamer, J.H. (1958). Total fatty acids in stool. Seligson D eds. Standard methods of clinical chemistry. Academic Press, New York. Pp. 34-39.

Vining, E.P.G. and Freeman, J.M. (1998). A multi-center study of the efficacy of the ketogenic diet. Archives of Neurology, 55: 433-1437.