Evaluation of Neurobehavioural and Cognitive Changes Induced by Carbamazepine and/or Phenytoin in Wistar Rats

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Received: 13 December 2015 Accepted: 3 January 2016 Published: 15 January 2016

Abstract

The study was to evaluate the neurobehavioural and cognitive changes in Wistar rats administered carbamazepine (CBZ), phenytoin (PHE) and their combination. Forty, apparently, healthy adult male Wistar rats weighing about 300 g were divided into four groups of 10 animals each. Group I rats were administered distilled water at 10 ml/kg. CBZ, 20 mg/kg, PHE 100 mg/kg and CBZ+PHE, 20 mg/kg and 100 mg/kg respectively were administered to groups II, III and IV, per os. The regimens were given once daily for eight weeks, the rats were monitored for neurobehavioural and cognitive changes. The results showed that administration of CBZ, CBZ+PHE and PHE decreased ($P < 0.05$) locomotion of the treated rats. Rearing decreased ($P < 0.05$) in rats treated with PHE. Cognition was not significantly affected by the treatments. In conclusion, chronic administration of CBZ, PHE and CBZ+PHE decreased locomotion, while PHE alone decreased rearing in Wistar rats.

Index terms— cognitive function, carbamazepine, phenytoin, locomotion, short-term memory, rearing.

1 I. Introduction

pilepsy is a disorder of the brain, characterized by an enduring predisposition to generate at least one seizure (Fisher et al., 2005). The term 'epilepsy' is usually restricted to those cases with a tendency for recurrent seizures (Nair, 2003). It has been reported that patients with epilepsy are at substantial risk of memory impairment, and results obtained from animal studies have demonstrated impaired hippocampal function as measured by spatial memory in rodents subjected to seizures (Zhou et al., 2007). Therefore, memory impairment is one of the neurobehavioural complications associated with epilepsy (Ali et al., 2003).

It was reported that rather than being overtly manifest, subtle changes in cognitive and psychomotor functions do occur commonly with long-term antiepileptic drug therapy, especially PHE sodium (Meador et al., 1991). However, recent findings indicate that rats with focal onset of spontaneous seizures respond to treatment following antiepileptic drug administration; but like humans, the responses to antiepileptic drugs can vary substantially between animals (Nissinen and Pitkanen, 2007). Polytherapy in epilepsy is a preferred treatment regimen in patients with intractable seizures (Macdonald and Meldrum, 1995). Rational polypharmacy of antiepileptic drugs is one of the treatment strategies for refractory epilepsy (Sun et al., 2002). Antiepileptic drugs, particularly those used in polytherapy, are the main causes of cognitive impairment in epileptic patients (Bernadi and ??arros, 2004). However, the effects of some of the drug combinations on neurobehavioural and cognitive changes have not been elucidated. Carbamazepine (CBZ) is an anticonvulsant used to treat epilepsy and mood disorders (Almgrem et al., 2008). It is administered alone or in combination with other medications to treat certain types of seizures in patients with epilepsy (Porter and Meldrum, 2007). Its main function is reduction of sustained repetitive firing in neurones by blocking voltage-gated sodium channels (Mathew et al., 2011). It also potentiates gammaaminobutyric acid (GABA) receptors (Granger et al., 1995). Thus, CBZ exerts therapeutic effects via inhibition of brain neuronal activities. The drug is widely used in Nigeria for the treatment of seizure disorders and trigeminal and other neuralgias (Shannon and Love, 2004). Phenytoin sodium (PHE), a hydantoin anticonvulsant, is one of the classical antiepileptic drugs (K?erk et al., 1998) and its systemic administration induces anticonvulsant effects in humans and experimental animals (Rykaczewska-Czerwi?ska, 2007). It is used widely in the treatment of generalized or partial seizures, except absence seizures (Vijay et al., 2009). It acts by blocking sodium channels and inhibits persistent sodium currents in neurones, thus inhibiting neuronal firing in the brain (Bryan and Waxman, 2005). It has also been shown to protect against axonal degeneration of spinal cord axons, and it improves neurological outcome of experimental allergic encephalomyelitis in mice (Luszcki, 2004). Unfortunately, none of the new antiepileptic drugs is superior in efficacy to the older drugs in terms of seizure remission (Shannon and Love, 2005).

2 II. Objective

The objective of the present study was to evaluate the effects of long-term administration of a combination of CBZ and PHE on neurobehavioural and cognitive changes in Wistar rats.

3 III. Materials and Methods

4 a) Animals

For the present study, 40 adult male Albino rats weighing between 144 and 300 g were used for the experiment. The animals were obtained from the animal house of the Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria and were housed in rat cages. The animals were given access to feed pellets made from growers' mash (Grand Cereals, Jos, Nigeria), maize bran and groundnut cake in the ratio 4:2:1, with wheat flour serving as binder, and water was provided ad libitum. The animals were allowed to acclimatize for a period of two weeks before the commencement of the experiment. The animals were divided at random into four groups of 10 animals each. Rats in groups II, III and IV were given CBZ (20 mg/kg), PHE (100 mg/kg) and CBZ+PHE (20 and 100 mg/kg separately), respectively. Rats in group I were given distilled water at 10 ml/kg and served as the untreated control. All treatments were administered orally by gavage once daily for a period of eight weeks. During this period, the rats were monitored for clinical and neurobehavioural signs.

5 b) Anticonvulsant drugs

The anticonvulsant drugs used in this study were CBZ tablets (Hovid Bhd, Malaysia) at 20 mg/kg (Rajesh et al., 1991) and PHE capsules (Biomedicine, Belgium) at 100 mg/kg (Vijay et al., 2009)

6 c) Evaluation of locomotor activity

The effect of the regimens on locomotor activity was evaluated weekly till the end of the experiment using the open-field apparatus (Zhu et al., 2001). The open-field apparatus was constructed using cardboard box (50 × 50 × 46 cm high) with clear Plexiglas on the floor. The floor of the box was divided into 25 equal squares. The locomotor activity was assessed by placing a rat in the box and allowing it to roam freely for 3 minutes to familiarize itself with the environment. The number of squares crossed with all the paws during the next 2 minutes was recorded. The arena was cleaned first with soapy water, followed by 90% alcohol solution to eliminate odours from the preceding animal.

7 d) Evaluation of rearing activity

Rearing activity was evaluated weekly till the end of the experiment, using the open-field apparatus (Zhu et al., 2001). Rearing was assessed by placing a rat in the box and allowing it to roam freely for 3 minutes to familiarize itself with environment. The number of times an animal stood on its hind limb trying to peep out of the box in the next 2 minutes was recorded. Soapy water followed by 90% alcohol solution was used to clean the arena.

8 e) Assessment of learning

This experiment was performed 48 hours prior to the termination of the study. It was done using the step-down inhibitory avoidance learning task as (Zhu et al., 2001). The apparatus used was made of 40 × 25 × 25cm acrylic chamber, consisting of a floor made of parallel 2 mm calibre stainless steel bars spaced 1 cm apart. An electric shock was administered through the floor bars. A 25cm high, 8cm by 25cm wooden platform was placed at the extreme end of the chamber. Each animal was placed gently on the platform; upon stepping down, the rat received a single 80 volts footshock. If the animal did not return to the platform, the foot-shock was repeated every 5 seconds. A rat was considered to have learned the avoidance task if it remained on the platform for more than 2 minutes. The number of foot-shocks applied before the animal learned the avoidance task was recorded as an index of learning acquisition.

9 f) Assessment of short-term memory

Memory was also assessed using the stepdown inhibitory avoidance task (Zhu et al., 2001). The apparatus used was the same as that described for learning. Briefly, individual rats were again placed gently on the platform

24 hours after performing the learning task. The time during which the animal remained on the platform was recorded as an index of memory retention. Staying of the rat on the platform for 2 minutes was counted as maximum memory retention (ceiling response).

The research was carried out according to the Ahmadu Bello University Animal Research Committee and in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication number 85-23), revised 1985, also according to the Guidelines for the use of animals in Neuroscience Research, 1992.

10 g) Statistical analysis

Values obtained were calculated as mean ± SEM and analysed using one-way analysis of variance (ANOVA).The differences between the variant means were separated using Tukey's post-hoc test. GraphPad prism version 4.0 for windows from GraphPad Software, San Diego, Carlifonia, USA (www.graphpad.com) was used for the analysis. Values of $P < 0.05$ were considered significant.

11 IV. Results

12 a) Effect of treatments on locomotion in Wistar rat

There were significant ($P < 0.05$) decreases in the number of squares crossed by rats in the CBZ and

13 b) Effect of treatments on rearing activity in Wistar rats

There was a significant ($P < 0.05$) decrease in rearing in the PHE group when compared to the control group. There was no significant ($P > 0.05$) change in the CBZ and CBZ+PHE groups when compared to control. Likewise rearing did not change when the treatment groups were compared. (Figure **??**). CBZ+PHE groups. The number of squares crossed by rats in the PHE group decreased significantly ($P < 0.01$), when compared to that of the control group, and no significant ($P > 0.05$) change in the number of squares crossed by rats was obtained when the treatment groups were compared (Figure **??**).

14 c) Effect of treatments on learning ability in Wistar rats

There was no significant ($P > 0.05$) change in the number of foot-shocks when the drug-treated groups were compared to the control group. Similarly, there was no significant ($P > 0.05$) change in the number of foot-shocks applied in between the treatment groups (Figure 3).

15 d) Effect of treatments on short-term memory in Wistar rats

There was no significant ($P > 0.05$) change in the duration of stay on the platform when the treatment groups were respectively compared to the control group. The changes observed were insignificant ($P > 0.05$) between the treatment groups (Figure 4).

16 V. Discussion

The decrease in locomotion recorded in the CBZ group agreed with the findings of (Luszcki, 2004), who reported a decrease in ambulatory activity and total distance covered in mice administered with CBZ. Nowakowska et al. (2011) also attributed the decrease in locomotor activity in the CBZ monotherapy in rats to the sedative effects of the drug, and suggested that this may be related to the induction of microsomal enzymes of the P 450 cytochrome.

The decrease in locomotion (Fig. **??**) observed with the polytherapy group agrees with the findings of Luszcki (2004) who reported that combining two sodium-channel blockers may result in a considerable reduction in locomotor activity of the animals tested; which, apparently, induced the potentiation rather than the summation of hypolocomotor effects produced by the combination of the antiepileptic drugs.

17 Rearing

Rearing reflects adaptive strategy of animals to explore their environment and also responses to environmental novelty and emotional states, such as stress levels in rodents (Ambali, 2009). Rearing responses observed upon repeated exposure to the same environment are strongly influenced by interindividual differences in habituation and this could be influenced by a variety of pharmacologic and toxicologic agents (Ambali, 2009).

The decrease in rearing activity (Fig. **??** finding may also be caused by the inhibition of calciuminduced secretory processes, including hormones and neurotransmitters released as a result of decrease in calcium permeability, with inhibition of calcium influx across the membrane (Porter and Meldrum, 2007). K?erk et al. (1998) reported that PHE modulates the direct activation of the motor system by stimulating the sensorimotor cortex in the adult, but not immature, rats.

18 VI. Conclusion

Chronic administration of CBZ and CBZ+PHE decreased locomotion but PHE alone decreased rearing in Wistar rats. Patients taking these drugs should therefore be adequate monitoring while taking their medications and if the aforementioned signs are noticed should be placed on alternative medications.

19 VII. Acknowledgement

Figure 1: E

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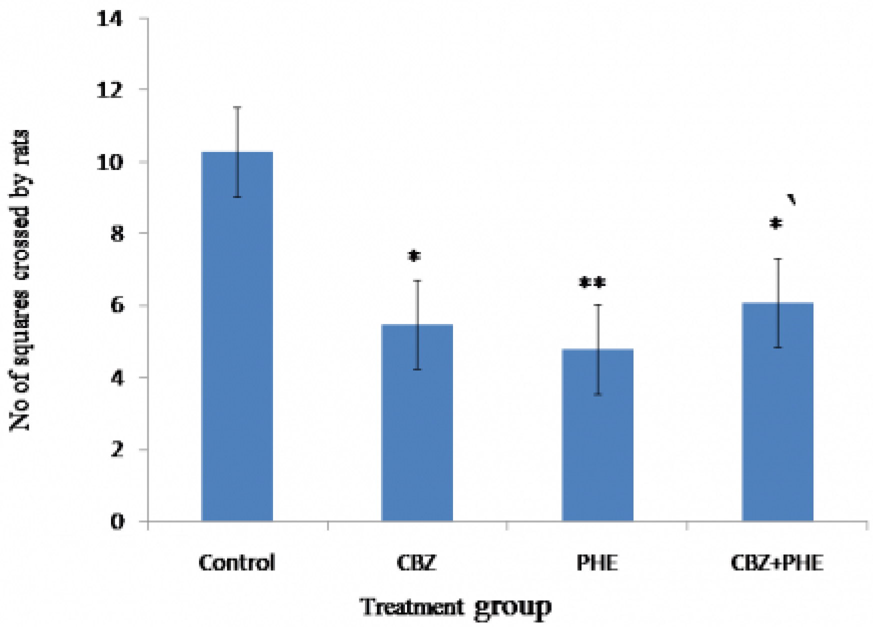

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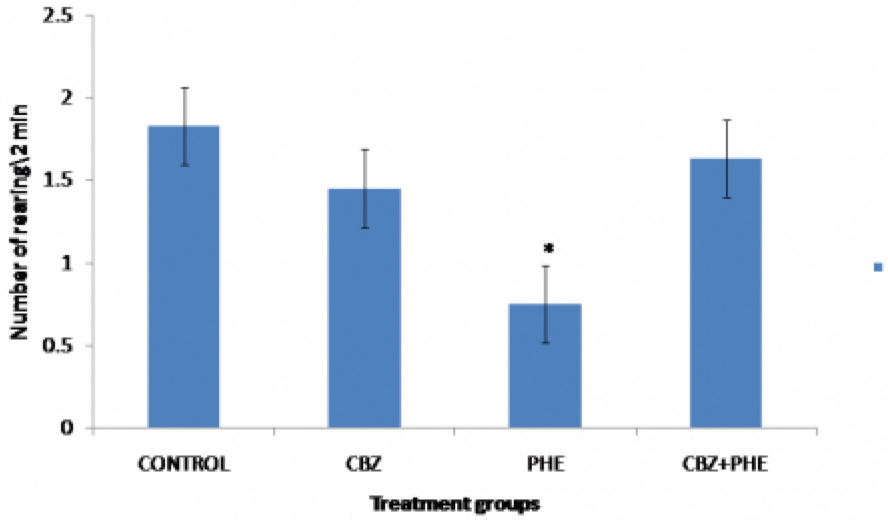
Figure 2: Figure 2 :Figure 1 :



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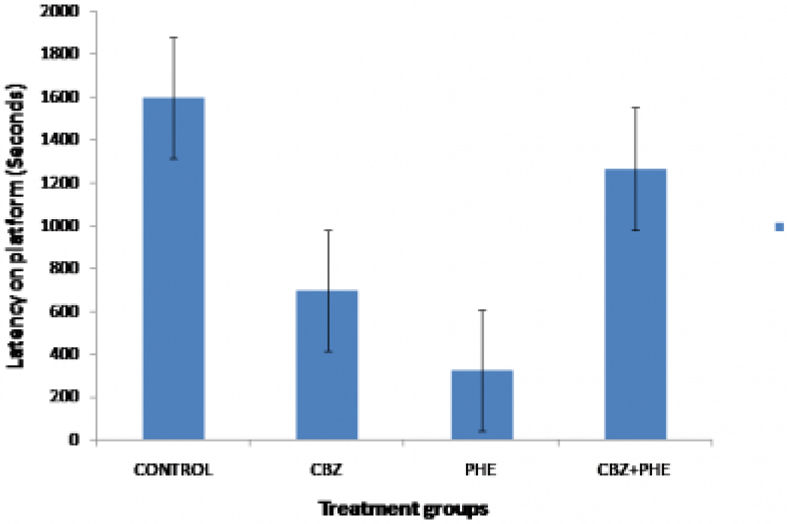

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Figure 4: Figure 4 :

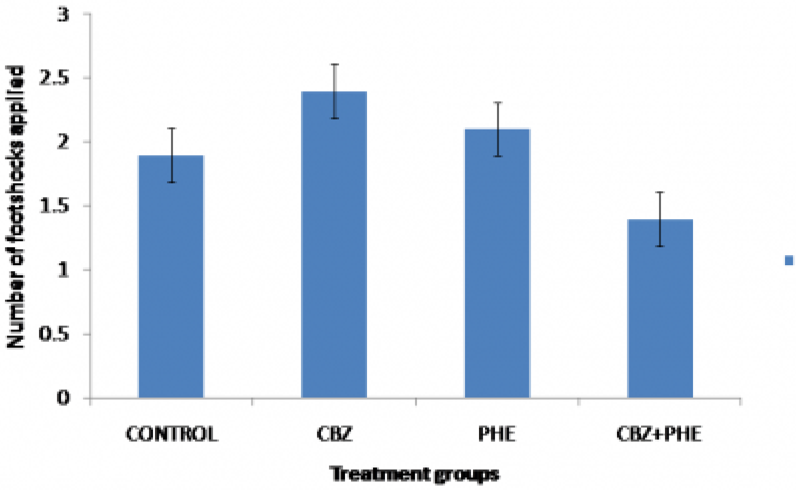
3
2.5
2
1.5
1
0.5
0
Number of footshocks applied
CONTROL
CBZ
PHE
CBZ+PHE
Treatment groups

Figure 5:

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