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L-Arginine Co-Administration with Carbamazepine Improves Cognition in Male Sprague-Dawley Rats

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Summary: Cognitive impairment is a common adverse effect associated with carbamazepine use. One of the proposed mechanisms for cognitive impairment may be attributed to the pro-oxidant properties of carbamazepine. This study investigated the effects of L-Arginine supplementation with carbamazepine on cognition in adult male non-epileptic rats. Adult male Sprague-Dawley rats with average weight 200g to 220g were divided into 4 groups; (1) Control group treated with distilled water, (2) L-Arginine group treated with L-Arginine (100mg/kg BW) in distilled water, (3) Carbamazepine group treated with carbamazepine (25mg/kg BW twice daily) in distilled water, and (4) Carbamazepine + L-Arginine group treated with Carbamazepine and L-Arginine as above for two weeks to assess the acute changes in cognition and oxidative stress markers. Following two weeks of treatment, cognition was assessed using the Y-maze, after which the rats were humanely sacrificed with the hippocampus and frontal lobes isolated from the brain and subsequently homogenized for assessment of oxidative stress markers [(Catalase, superoxide dismutase (SOD), malondialdehyde (MDA), and reduced Glutathione (GSH)]. Arm entry and correct alternation were significantly higher ($p < 0.05$) in the L-Arginine and L-Arginine + Carbamazepine groups compared to carbamazepine group. In the frontal lobe, L-Arginine significantly increased ($p < 0.05$) catalase and GSH levels compared to other groups while in the hippocampus**,** it significantly ($p < 0.05$) reduced MDA with no change in other parameters. Likewise, SOD and MDA levels were significantly lower ($p < 0.05$) in the L-Arginine + Carbamazepine group compared to other groups. Oral L-Arginine supplementation with carbamazepine improved cognitive performance on Y maze.

Keywords*:* Carbamazepine, L-Arginine, Cognition, Oxidative stress

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INTRODUCTION

Carbamazepine is one of the most commonly prescribed antiepileptic drugs and also useful in the management of non-epileptic disorders. It is a first line drug in the management of focal seizures however, its negative effect on cognitive function has been well documented (Wesnes *et al.*, 2009; Gulec Suyen *et al.*, 2016). In addition, cognitive impairment is commonly reported in epileptic seizures (Mula and Trimble, 2009). Occurrence of cognitive impairment is usually more pronounced on drug introduction and during upward titration of drug dosage. As a result of this, patients may be poorly adherent or become non-adherent to drug therapy with a consequent suboptimal seizure control (Perucca and Gilliam, 2012). Evaluating the mechanism and interventions for early cognitive changes will help to address this.

Carbamazepine acts by inhibiting voltage gated sodium channels but its effect on cognition may not be related to its mechanism of action because lamotrigine which acts through a similar mechanism is not linked to negative cognitive impacts (Arora *et al.*, 2010). In fact, some studies have reported an improvement in cognitive profile following lamotrigine use (Shinnar *et al.*, 2017; Zhang *et al.*, 2017).

Generation of oxidative stress following carbamazepine use has been reported as the basis for the development of cognitive impairment (Arora *et al.*, 2010; Reeta *et al.*, 2010). Oxidative stress is a common phenomenon which occur due to a failure in the normal balance between production and the mopping up of reactive oxygen (ROS) and nitrogen species (RNS) which leads to the disruption of structural integrity and normal function of the lipid membrane, protein and the DNA (Ullrich and Kissner, 2006). The major source of these ROS is from the mitochondrial respiratory chain and nitric oxide synthase activity. Reactive species responsible for oxidative stress are superoxide O_2^-, hydrogen peroxide H_2O_2, hydroxyl radicals OH^- and other nitrogen containing radicals such as NO^-, NO^+, NO_2^-, ^-OONO (Ullrich and Kissner, 2006). Superoxide (O_2-) and H_2O_2 are generated from mitochondrial respiration, they undergo further enzymatic reactions

to produce other reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Egea *et al*., 2017).

Cellular and tissue defences against ROS and RNS include the enzymes superoxide dismutase catalase, glutathione peroxidase, peroxiredoxins and the nonenzymatic antioxidants, glutathione (GSH), thioredoxin, ascorbate, α-tocopherol and uric acid (Egea *et al.*, 2017). The levels and activities of these are used to determine oxidative stress. The extent of tissue and cellular damage from oxidative stress is obtained by measuring the markers of lipid peroxidation such as malondialdehyde, 4-hydroxy-alkenals, thiobarbituric-reactive substances (TBARS) and DNA damage like 8-Hydroxyguanine (8-OHdG) and 8-Hydroxy-2'-Deoxyguanosine (oH8DG) (Silva *et al*., 2014).

Oxidative stress has been shown to affect the synthesis and release of neurotransmitters which are critical components of cognition (Waldbaum and Patel, 2010). Carbamazepine has been associated with oxidative stress in both animal and human studies (Reeta *et al.*, 2010; Tutanc *et al.*, 2015). This suggest that anti-oxidants may be helpful in ameliorating the effect of carbamazepine on memory. An earlier study has demonstrated the benefit of a natural antioxidant, curcumin in cognitive impairment associated with carbamazepine use (Reeta *et al.*, 2010). It has also been stated that L-Arginine subserves positive cognitive impacts by increasing the levels of glutathione and inhibiting the release of superoxide (Liang *et al*., 2018). This is because of its ability to increase the level of nitric oxide in the prefrontal cortex and hippocampus, both areas of which are important for cognitive improvement (Wei *et al.*, 2013; dos Santos *et al*., 2014).

Similar to the somatotopic organisation of the brain, the hippocampus and amygdala mediate memory formation, while processing speed and executive functions are localized to the prefrontal lobes, although, there is an overlap in the function of various regions (Purves, 2012). A large number of studies have reported the effects of carbamazepine on cognitive function, however there is paucity of data on how to prevent this cognitive adverse effect. Thus, this study investigated the role of L-Arginine supplementation on early cognitive changes, oxidative stress indices, in both the frontal and hippocampus and their relative contribution to cognitive changes.

MATERIALS AND METHODS

Animals

Healthy adult male Sprague-Dawley rats (average weight 200g to 220g) were obtained from the Animal House of the College of Medicine, University of Lagos. The animals were housed in polypropylene cages (30 x 15 x 15cm) in groups of eight rats per cage with free access to pelletized rat chow and water *ad libitum.* Rats were kept under normal light conditions (12 hours light/dark cycle) and normal room temperature (24 ± 2 °C). All experiments were carried out during the light phase between 9:30 a.m. to 12.00noon and performed in accordance with the international regulations to minimise pain on laboratory animals. Our research protocol was also in line with the guidelines of the College of Medicine, University of Lagos, Health Research and Ethics Committee.

Drugs and dosing schedule

Carbamazepine (Tegretol) was obtained from Novartis Pharma AG, Basle, Switzerland while L-Arginine was obtained from Now Foods, Bloomingdale, USA. Carbamazepine was administered at a dose of 25mg/kg twice daily and L-Arginine (100mg/Kg) daily both for 2 weeks. This dose for carbamazepine was used based on reports from previous study (Nissinen and Pitkanen, 2007). The control animals received distilled water daily for the same period of study. On the final day of experiment, both drugs were administered an hour prior to cognitive assessment in the animals.

Assessment of cognition

Cognition was assessed in the rats at 2 weeks following carbamazepine treatment and L-Arginine supplementation. The animals in all groups were pre-exposed to the Y maze twenty-four hours before the experiment and were brought to the laboratory at least two hours before the experiments for acclimatization on the day of the experiment.

Y-Maze

The Y- maze is composed of three equally spaced arms (120°, 41cm long and 15cm high). The floor of each arm is 5cm wide. The Y-maze is used to assess short term spatial memory, general locomotor activity and stereotypic behaviour. The Y-maze is used to assess cognitive function in animals (Ishola *et al.*, 2017). The ability to have correct alternation in the maze is a measure of working memory which includes short term memory, attention and information processing. This requires an unaltered signally pathway between the hippocampus and the prefrontal cortex (Morellini, 2013).

Parameters measured in this study using the Y maze included number of arm entry and percentage correct alternation. Each rat was placed at the centre of the Y-maze and the sequence of arm entries was documented, and percentage correct alternation calculated. A correct alternation is defined as entry into all three arms consecutively. The Y-maze assessment was carried out for 4 minutes. The apparatus was cleaned with 5% alcohol and allowed to dry between sessions.

Brain tissue for oxidative stress assessment

The rats were humanely sacrificed by using a standard cervical dislocation procedure. The brains were quickly isolated with hippocampus and frontal lobe

dissected from the brain, weighed, washed in ice-cold normal saline and homogenized in 10% ice-cold 0.1 M potassium phosphate buffer (pH 7.4). The homogenates were centrifuged at 3000 RPM for 15 minutes in a cold centrifuge with supernatants separated and stored at -70^{o} C for the measurement of oxidative stress markers [(Superoxide dismutase (SOD), Malondialdehyde (MDA) and reduced glutathione (GSH), and Catalase levels)] as described in a previous study (Arora *et al*., 2010).

Glutathione level was estimated using the method described by Ellman (Ellman, 1959). Catalase activity was determined by the colorimetric method as previously described (Clairborne, 1985). Malondialdehyde was estimated using the method described by Ohkawa and co-workers (Ohkawa *et al.*, 1979), and Superoxide dismutase activity was determined by the pyrogallol auto-oxidation method described by Marklund (Marklund, 1985).

Statistical Analysis

All results were expressed as mean ± S.E.M of rats 8 in each group. The data were statistically compared by carrying out One-way Analysis of Variance (ANOVA) using GraphPad Prism 7 followed by post-hoc Tukey's test for inter-group comparisons. A value of $p < 0.05$ was considered statistically significant for comparison.

RESULTS

Assessment of Cognition

The number of arm entry and percentage correct alternation were significantly lower ($p < 0.05$) in the carbamazepine group compared to control. L-Arginine and CBZ + L-Arginine groups had significantly higher arm entry and percentage correct alternation ($p < 0.05$) compared to control and the carbamazepine group (Figures 1 and 2).

Glutathione levels

In the frontal lobe, GSH level was significantly higher ($p < 0.05$) in the L-Arginine group compared to control, carbamazepine and CBZ + L-ARG groups. Likewise, CBZ + L-ARG group had significantly increased GSH level ($p < 0.05$) compared to carbamazepine but no significant difference compared to control. In the hippocampus GSH level was significantly lower ($p < 0.05$) in the carbamazepine group compared to control while it was significantly higher ($p < 0.05$) in the L-Arginine group compared to control and CBZ +L-ARG groups (Figure 3).

Catalase

In the frontal lobe, catalase level was significantly higher ($p < 0.05$) in the L-ARG and CBZ + L-ARG groups compared to control and CBZ groups while there was no significant difference in the levels of catalase in the CBZ group compared to control. In the hippocampus there was no significant difference in catalase levels in all the groups (figure 4).

Superoxide dismutase

In the frontal lobe, SOD concentration was significantly higher ($p < 0.05$) in the CBZ group compared to the others groups. However, in the hippocampus, CBZ + L-ARG group had significantly

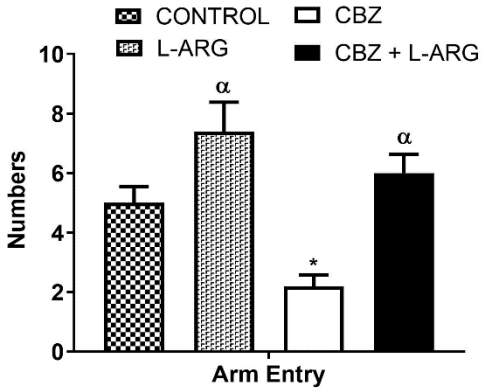

Figure 1. The effect of L-arginine co-administration with carbamazepine on Arm entry in Control, L-ARG, CBZ, and CBZ + L-ARG rats. *P < 0.05 Vs. Control., αP < 0.05 Vs CBZ.

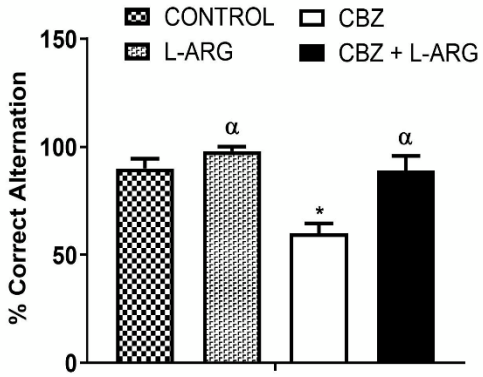

Figure 2. Percentage Correct alternation in Control, L-ARG, CBZ, and CBZ + L-ARG rats following L-Arginine co-administration with carbamazepine.
*P < 0.05 Vs. Control., αP < 0.05 Vs CBZ.

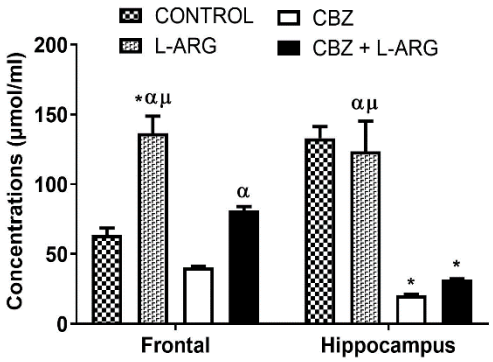

Figure 3. The effect of L-Arginine co-administration with carbamazepine on GSH concentrations in Frontal lobe and Hippocampus in Control, L-ARG, CBZ, and CBZ + L-ARG rats.*P < 0.05 Vs. Control., αP < 0.05 Vs CBZ., µP < 0.05 Vs CBZ + L-ARG

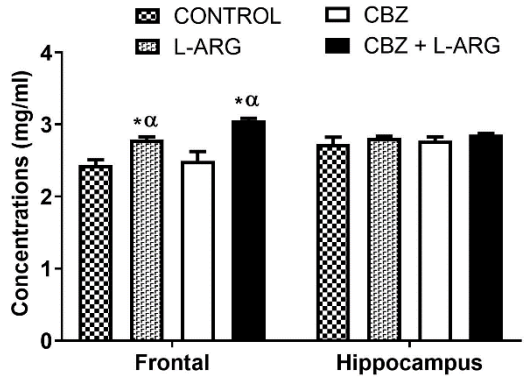

Figure 4. Catalase concentration in Control, L-ARG, CBZ, and CBZ + L-ARG rats in Frontal lobe and Hippocampus following L-Arginine co-administration with carbamazepine. *P < 0.05 Vs. Control., αP < 0.05 Vs CBZ.

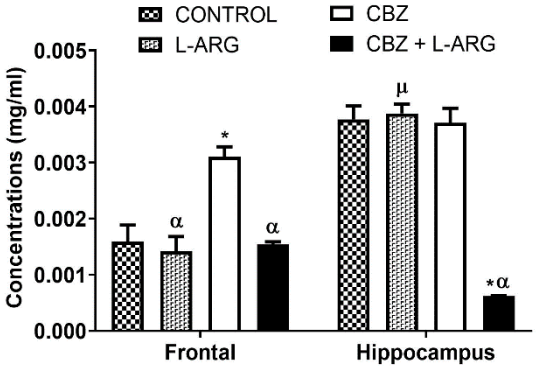

Figure 5. The effect of L-arginine co-administration with carbamazepine on SOD concentrations in Frontal lobe and Hippocampus in Control, L-ARG, CBZ, and CBZ + L-ARG rats. *P < 0.05 Vs. Control., αP < 0.05 Vs CBZ., μP < 0.05 Vs CBZ + L-ARG

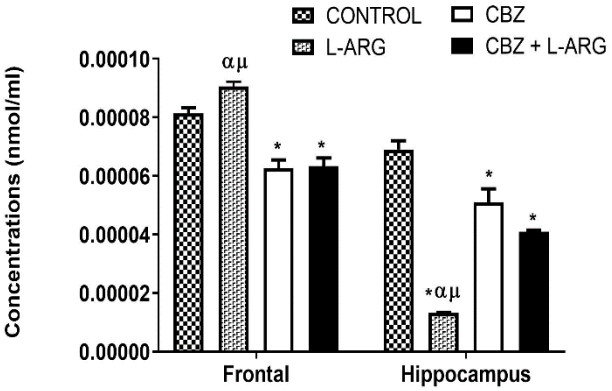

Figure 6. The effect of L-arginine co-administration with carbamazepine on MDA concentrations in Frontal lobe and Hippocampus in Control, L-ARG, CBZ, and CBZ + L-ARG rats.*P < 0.05 Vs. Control., αP < 0.05 Vs CBZ., μP < 0.05 Vs CBZ + L-ARG

lower (p < 0.05) level of SOD compared to the other groups (figure 5).

MDA concentration

In the frontal lobe, CBZ and CBZ + L-ARG groups had significantly lower (p < 0.05) levels of MDA compared to control and L-ARG groups. Likewise, in the hippocampus, MDA was significantly lower (p < 0.05) in the CBZ group compared to controls. While the L-ARG and CBZ + L-ARG groups also had significantly lower (p < 0.05) levels of MDA compared to the control group (Figure 6).

DISCUSSION

The study showed that carbamazepine reduced the total number of arm entry, number of alternation and percentage correct alternation in the Y maze compared to controls. These findings support previous studies demonstrating the negative impact of carbamazepine on different cognitive domains in both human and animal studies (Garcia-Penas *et al*., 2014; Xiao *et al*., 2018; Gulec Suyen *et al*., 2016; Olusanya et al., 2017).

Carbamazepine was shown to impair spatial learning and attention in non-epileptic rats (Shannon and Love, 2004; Shannon and Love, 2005; Shannon and Love, 2007; Barker-Haliski *et al*., 2016). However a few studies have reported improvement in cognitive function or no changes following carbamazepine therapy (Bernardi and Barros, 2004). Most of the study reporting improvement are limited by the fact that studies were carried out in epileptic rats and reduction in seizure frequency may contribute to the improvement in cognition rather than the direct effect of carbamazepine (Bernardi and Barros, 2004).

To the best of our knowledge, this is one of the few studies that have addressed the cognitive dysfunction associated with carbamazepine use. The group administered with L-Arginine alone and carbamazepine + L-Arginine had improved parameters which suggests that L-Arginine improves cognition and its supplementation is protective against cognitive impairment associated with carbamazepine use. Studies have shown the association between improved cognition and the use of L-Arginine.

L-Arginine is a semi-essential amino acid which participates in multiple biochemical processes in the body. It is a substrate for the formation of nitric oxide (NO) which serves as a neurotransmitter mediating the release of glutamate, GABA and dopamine (Sardo and Ferraro, 2007). NO activates guanylyl cyclase to produce cGMP, a mediator of neuronal plasticity and excitability (Flynn *et al.*, 2002; Gornik and Creager, 2004). Reduced nitric oxide has been linked to cognitive impairment (Katusic and Austin, 2014; Morita *et al.*, 2014). L-Arginine has been shown to increase the level of NO in the prefrontal cortex and hippocampus which correlates with cognitive improvement (Wei *et al*., 2013; dos Santos *et al.*, 2014).

Similarly, it has also been associated with increase in the expression of α7 nAChR immunoreactivity and protein expression in the prefrontal cortex and hippocampus which are all associated with improved cognitive function in animal studies (Wei *et al.*, 2013). Alpha 7- nicotinic receptors are located mainly on presynaptic neurones on the hippocampus, amygdala

and prefrontal cortex where they regulate the release of neurotransmitters like GABA and glutamate, and on postsynaptic neurons where they mediate fast excitatory neurotransmission (Sinkus *et al.,* 2015; Kalkman and Feuerbach, 2016). The anti-oxidant and anti-inflammatory properties of L-Arginine may also explain its beneficial effect on cognition (Fonar *et al.,* 2018) since both inflammation and oxidative stress have been implicated in cognitive impairment (Magenta *et al.,* 2014).

One of the possible mechanism for the development of cognitive impairment with carbamazepine use is due to its pro-oxidative capability which has been demonstrated in several studies (Reeta *et al*., 2010; Li *et al*., 2010; Tutanc *et al*., 2015). Oxidative stress is implicated in the disruption of both glutamatergic and cholinergic neurotransmission (Waldbaum and Patel, 2010).

In this study, carbamazepine significantly reduced the levels of glutathione in the hippocampus and this supports the pro-oxidant capacity of carbamazepine as previously documented (Reeta *et al.,* 2010). Other studies have reported no change in the levels of glutathione following the administration of carbamazepine (Yuksel *et al.,* 2000, Arora *et al.,* 2010). Various studies have demonstrated conflicting reports on SOD levels in relation to carbamazepine use. For example, some studies have reported an increase in SOD levels (Liu *et al.,* 1998, Yuksel *et al.,* 2001) following carbamazepine administration, while other studies have reported a reduced or an unchanged SOD levels (Yuksel *et al.,* 2000, Verrotti *et al.,* 2002) following carbamazepine administration. Our study showed a significant increase in SOD levels in frontal lobe in the CBZ group compared to the other groups.

The source of oxidative stress following carbamazepine use is unknown, but may be related to its metabolism via cytochrome P450 system. This needs to be confirmed in further studies. A few studies however did not find any effect on oxidative stress parameter in non-epileptic rats and humans (Arora *et al.,* 2010; Menon *et al.,* 2014). A study on human subjects reported no difference in oxidative stress parameters in patients on antiepileptic drugs which included carbamazepine and drug naïve patients with epilepsy. The study concluded that oxidative stress is secondary to the presence of seizures not the use of antiepileptic drugs (Menon *et al.,* 2014).

This study showed different oxidative stress pattern in the frontal lobe compared to the hippocampus (Figures 3 - 6). This pattern of differential markers of oxidative stress with relation to location and timing has been demonstrated in different studies (Freitas *et al*., 2005; Bellissimo *et al.,* 2001). Freitas *et al.,* (2005) suggested that catalase/and/or GSH as the major radical scavenging system in the hippocampus because their levels were more likely to be reduced following oxidative stress.

The outcome following the use of antioxidants and conditions associated with cognitive dysfunction such as Alzheimer's disease and epilepsy have been conflicting (Skvarc *et al.,* 2017; Farina *et al.,* 2017; Tarantini *et al.,* 2018). A recent study demonstrated that treatment of status epilepticus with N-acetylcysteine and sulforaphane; drugs that increase the levels of GSH, was protective against oxidative stress and cognitive decline (Pauletti *et al*., 2017). Only a few studies addressed the use of antioxidants in drug-induced cognitive dysfunction. Reeta et al. (2010) reported that Curcumin, a compound derived from plants was shown to pevent cognitive dysfunction in rats administered carbamazepine and this was linked to its anti-oxidants potential.

L-Arginine improved oxidative parameters in this study. This suggests that improved cognitive function in the rats on L-Arginine may be related to its anti-oxidative properties, though other mechanisms like increase in nitric oxide synthesis and alpha 7 nicotinic acetylcholine receptors activation have been linked to its positive cognitive effect (Wei *et al.,* 2013; dos Santos *et al.,* 2014).

Our study demonstrated improved cognitive function in the carbamazepine + L-Arginine group compared to the other groups in non-epileptic rats but this is unlikely due to direct effect of oxidative parameters. Thus, further study in epileptic rats with a possibility to explore other mechanisms of actions like the effect on neurotransmission, and anti-oxidant protein activity in this improved cognitive process is suggested.

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