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Effects of Coadministration of Vitamin C and Lamotrigine on some Pharmacological Properties of Lamotrigine in Laboratory Animals

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ABSTRACT

Despite the continued development and release of new antiepileptic drugs, many patients have seizures that do not respond to drug therapy or have related side effects that preclude continued use. The present study is aimed at establishing the possible potentiation actions of vitamin C on the anticonvulsant effects of lamotrigine in acute and chronic seizure models and on the possible prevention and/or reversal of haematological side effects of lamotrigine. This aim was achieved using maximal electroshock test (MEST)-induced seizure model for which the method of Swinyard and Kupferberg, (1985) was adopted, pentylenetetrazole-induced kindling model for which method described by Gupta *et al*., (2001) was employed and haematological analysis in which haemoglobin concentration, packed cell volume, red blood cell count, white blood cell count / differentials and platelets count were carried out. Lamotrigine and Vitamin C combination at doses of 50 mg/kg, 100 mg/kg and 400 mg/kg provided 16.67%, 50%, and 66.67% protection respectively against MEST-induced seizures. There was no significant ($p \leq 0.05$) difference in the mean recovery period. Lamotrigine only showed 0% protection against MEST-induced seizures. In PTZ induced kindling, Vitamin C (50 mg/kg and 400 mg/kg) showed a significant ($p \leq 0.05$) reduction (from 5.00 to 0.88 and 5.00 to 2.13 respectively) in the severity of seizure induced by sub-convulsive dose (35 mg/kg) of PTZ as compared to control group on day ten. Administration of lamotrigine caused a reduction in mean neutrophils count as against untreated control group. Addition of vitamin C (50 mg/kg and 100 mg/kg) to lamotrigine treated group caused an increase in neutrophils compared to lamotrigine only treated group. The results showed that vitamin C enhances the anticonvulsant and some haematological sideeffects of lamotrigine, thus may serve as an adjunct in treatment of epilepsy using lamotrigine.

Key words: Lamotrigine, vitamin C, epilepsy, maximal electroshock-induced seizures, Pentylenetetrazole-induced kindling.

INTRODUCTION

Brain dysfunctions, whether primary or secondary to malfunction of other systems, are a major concern of human society, and a field in which pharmacological intervention plays a key role (Rang *et.al*., 2007). For a long time it was assumed that a single drug could be developed for the treatment of all forms of epilepsy, but the causes of epilepsy are extremely diverse, encompassing genetic and developmental defects, infective, traumatic, neoplastic and degenerative disease processes, thus drug therapy to date shows little evidence of aetiologic specificity (Porter and Meldrum, 2004). Treatment with standard anticonvulsants such as phenytoin, carbamazepine, valproic acid and phenobarbital is often complicated by side effects and by failure to adequately control seizures (William *et al*., 1998). Approximately, 20 to 30% of patients are refractory to therapies using currently available antiepileptic drugs (AEDs), (Sasa, 2006). Also, most AED_S are used over a long period of time and about 88 % of such drugs are associated with serious side effects e.g. renal failure, agranulocytosis and haemolytic anaemia (Baker *et al*., 1997). Vitamins have been considered to be important agents in controlling certain types of seizures or even preventing adverse effects of AEDs (Ranganathan and Ramaratnam, 2009). An indication for vitamin supplementation in epilepsy is where a vitamin may reduce seizure frequency through a presumed anticonvulsant role, possibly "by resetting'' the inhibitory gamma butyric acid – GABA and excitatory glutaminergic system. Vitamin E, folic acid and pyridoxal phosphate may have a role in all of these potential mechanisms (Keen and Appleton, 2006).

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Vitamin C, easily transported through the blood brain barrier, is proved to reduce injury in the hippocampus during seizures. Depending on type of seizure, it has mostly inhibitory activity and even decreases mortality (Edyta and Stanislaw, 2014). Vitamin C also enhances the absorption of iron. Iron helps make haemoglobin, the part of red blood cell that carries oxygen, thus aids in red blood cell production and its deficiency can lead to anaemia, or low red blood cell count (Loscher and Schmidt, 2006). So far, recommendations for the routine use of vitamin C for the treatment of epilepsy are yet to be made. It is therefore pertinent to widen the scope of therapy to include vitamin C with potentials of improving some pharmacological properties of lamotrigine. In this investigations, the effects of vitamin C on the anticonvulsant activity and haematological side effects of lamotrigine in laboratory animals was studied.

MATERIALS AND METHODS

Chemicals/Drugs and Equipment

Lamotrigine (Sigma Aldrich Chemical Co., USA), Vitamin C (Sigma Aldrich Chemical Co., USA), Pentylenetetrazole (Sigma Aldrich Chemical Co., USA), Normal Saline and Electroconvulsive machine (UgoBasile, Model No. 7801).

Animals

Adult Swiss Albino mice of both sexes (16-24g) and wistar rats of both sexes(150-200g) were obtained from the Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were kept under well-ventilated conditions at ambient temperature with constant supply of standard animal feeds and water *ad libitum*. The animals were allowed to acclimatize with the laboratory environment prior to the commencement of the experiment. Approval for the study was obtained from committee on animal use and care, Ahmadu Bello University Zaria. The approval number is ABUCAUC/2016/046.

Maximal electroshock test in mice

For maximal electroshock test, the method of Swinyard and Kupferberg, (1985) was adopted. Twenty four mice were randomly divided into four groups of six each. Group 1 received normal saline (10 mls/kg) p.o, daily for 5 days. Groups 2, 3, and 4, received vitamin C at dose of 50 mg/kg, 100 mg/kg, and 400 mg/kg (p.o) respectively for five days. On the 5^{th} day, 7.5 mg/kg (i.p) lamotrigine was administered to all the groups 30 minutes after vitamin C/normal saline administration. Subsequently, maximal electroshock was administered to induce seizure in the mice using UgoBasile electroconvulsive machine (Model 7801) with the electrodes placed on the pinnea of the mice. The current, shock duration, frequency and pulse width were maintained at 50 mA, 0.4 s, 50 pulse/s and 0.6 ms respectively. Episodes of tonic hind limb extension (THLE) were observed and recorded. An episode of THLE of the mice was considered as full convulsion while lack of THLE was regarded as protection.

Pentylenetetrazole–Induced Kindling in Rats

Method described by Gupta *et al*., (2001) was employed. Fifty six rats were divided into seven groups of eight each.

Group 1 received 1 ml/kg of oral (p.o) normal saline daily for 20 days.

Group 2 received 1 ml/kg of oral normal saline daily for 20 days and 35 mg/kg i.p PTZ every 48hrs for 20 days.

Group 3 received 1 ml/kg of oral normal saline daily for 20 days and 20 mg/kg i.p lamotrigine every 48hrs for 20 days.

Group 4 received 1 ml/kg of oral normal saline daily for 20 days and 20 mg/kg i.p lamotrigine every 48hrs. For each lamotrigine administration, 35 mg/kg i.p PTZ was served, also for a period of 20 days.

Groups 5,6, and 7 received 50 mg/kg, 100 mg/kg, and 400 mg/kg respectively of oral vitamin C daily for 20 days and 20 mg/kg i.p lamotrigine every 48hrs for 20 days. On alternate days, 35 mg/kg i.p PTZ was administered to rats in groups 5,6 and 7 for 20 days. Following each PTZ administration, the rats were observed for a period of 30 minutes and seizure intensities were scored on every treatment day as follows: Stage 0- no response, Stage 1- ear and facial twitching, Stage 2- convulsive waves throughout the body, Stage 3- myoclonic jerks, rearing, Stage 4- turning over on to one side, Stage 5- turning over on to the back, generalized tonic-clonic seizure.

Haematological Analysis

For this study, packed cell volume (PCV), Haemoglobin (HB) concentration, red blood cell (RBC) count, white blood cell (WBC) count and differentials and platelet count (PTC) were carried out.

Blood sample for these tests was taken through retro-orbital puncture from the rats at the end of PTZ-induced kindling in rats. All samples were collected in heparinized sample bottles and then taken to department of human anatomy Ahmadu Bello University, Zaria for the laboratory analysis.

Statistical Analysis

Statistical analysis of the data was carried out using SPSS (Version 20) and data obtained were expressed as Mean ± Standard Error of the Mean (SEM) in tables and figures as appropriate. The difference between means was analyzed using one way analysis of variance (ANOVA). Values of $p \leq 0.05$ where applicable were considered statistically significant.

RESULTS

Maximal Electroshock-induced Seizures in Mice

Lamotrigine and Vitamin C combination at doses of 50 mg/kg, 100 mg/kg and 400 mg/kg provided 16.67%, 50%, and 66.67% protection respectively against MEST-induced seizures. There was no significant ($p \leq 0.05$) difference in the mean recovery period. Normal saline showed 0% protection against MEST-induced seizures while vitamin C at doses of 50 mg/kg 100 mg/kg and 400 mg/kg showed 0%, 16.67% and 16.67% mortality respectively. (Table 1)

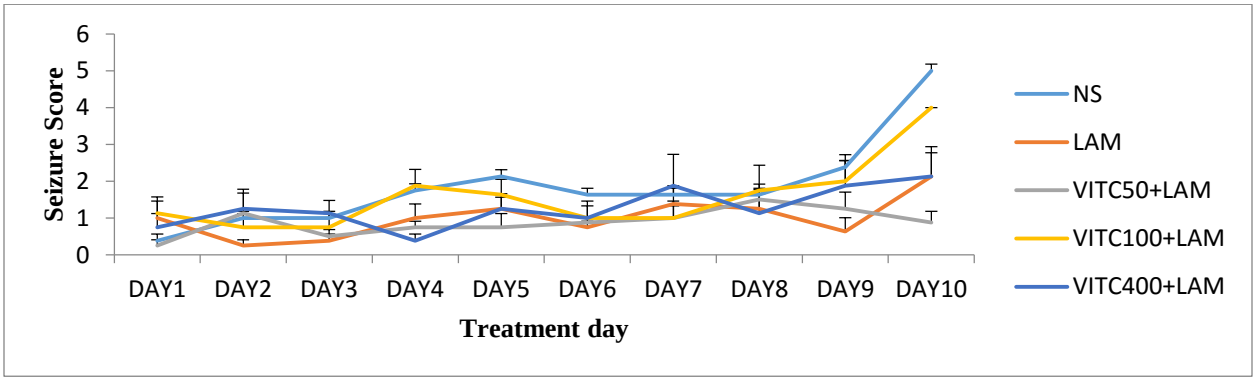
Table 1: Effects of vitamin C and Lamotrigine Co-administration on Maximal Electroshock-induced seizures in mice.

Treatment (mg/kg)	Mean Recovery Period (Sec)	Quantal protection	% Protection against seizure	Mortality	(%) Mortality
NS (10 ml/kg) + LAM (20)	24.50 ± 6.62	0/6	0.00	4/6	66.67
VitC (50) + LAM (20)	32.40 ± 4.49	1/6	16.67	0/6	0.00
VitC (100) + LAM (20)	18.00 ± 4.93	3/6	50.00	1/6	16.67
VitC400 + LAM (20)	-	4/6	66.67	1/6	16.67

Values are presented as mean ± SEM, No significant ($p \leq 0.05$) difference between control (N/S) group and other test groups. One way ANOVA. N=6, NS- Normal saline, VitC- vitamin C, LAM-Lamotrigine.

Pentylenetetrazole-induced Kindling in Rats (PTZ-Iinduced Iindling in Rats)

In PTZ-induced kindling model, vitamin C at doses of 50 mg/kg, and 400 mg/kg showed a significant ($p \leq 0.05$) reduction in the severity of seizure induced by sub-convulsive dose (35mg/kg) of PTZ as compared to control group on day ten. (Figure1)



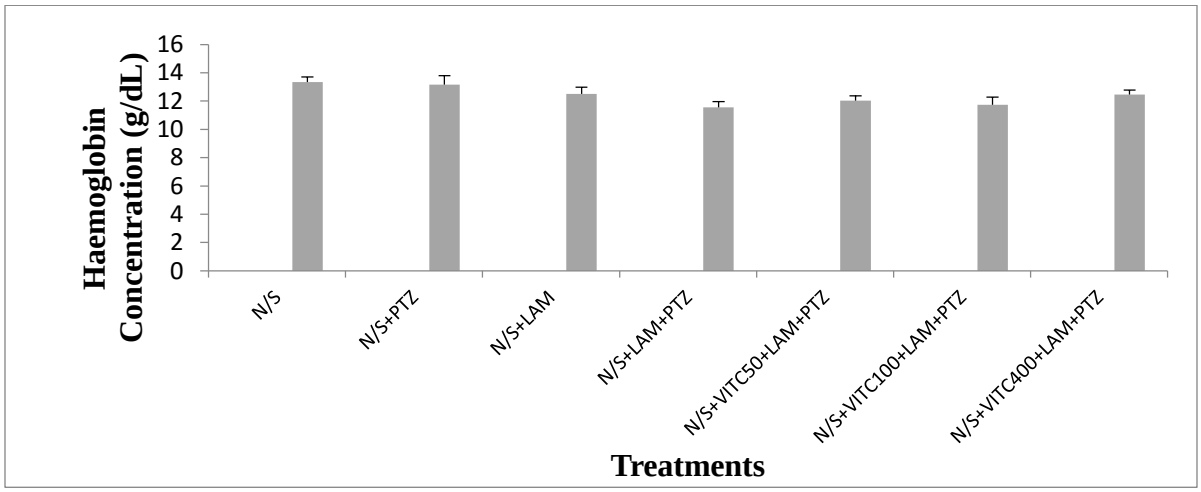
Seizure score presented as Mean ± SEM, superscript represent $p< 0.05$ compared to normal saline control group. Non parametric Kruskal-Wallis test, n=8, NS = Normal Saline, LAM = Lamotrigine, VITC = Vitamin C, PTZ = Pentylenetetrazole

Fig. 1: Effects of Vitamin C and Lamotrigine Co-administration on Pentylenetetrazole-induced Kindled Rats

Haematological Studies

Effects of Vitamin C and Lamotrigine Co-administration on Haemoglobin Concentration in Pentylenetetrazole-induced Kindled Rats

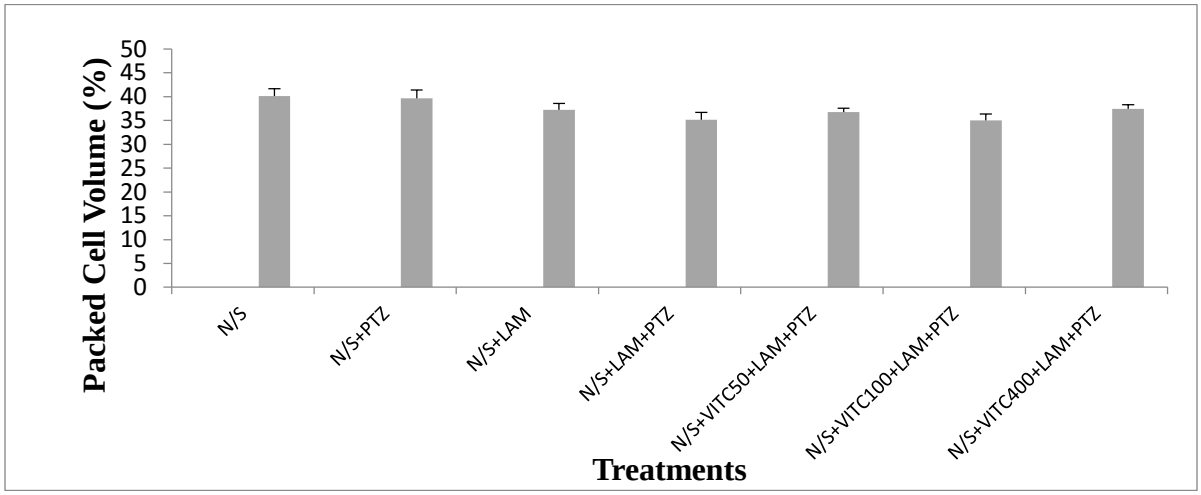
There was no difference in haemoglobin concentration in pentylenetetrazole kindled rats treated with lamotrigine and lamotrigine only (no PTZ treatment) groups compared to normal saline control group. Other groups additionally treated with vitamin C (50 mg/kg, 100 mg/kg, and 400 mg/kg) also showed no difference in haemoglobin concentration compared to the pentylenetetrazole kindled rats group treated with lamotrigine only. (Figure 2)



Values are presented as Mean ± SEM. One way ANOVA, no statistically significant difference when test groups are compared against N/S control group. n = 8, HB = Haemoglobin concentration NS = Normal Saline, VITC = Vitamin C, PTZ=Pentylenetetrazole, LAM=Lamotrigine.
Fig 2: Effects of Vitamin C and Lamotrigine Co-administration on Haemoglobin Concentration in Pentylenetetrazole-induced Kindled Rats

Effects of Vitamin C and Lamotrigine Co-administration on Packed Cell Volume in Pentylenetetrazole-induced Kindled Rats

There was no difference in packed cell volume in pentylenetetrazole kindled rats treated with lamotrigine and lamotrigine only (no PTZ treatment) groups compared to normal saline control group. Other groups additionally treated with vitamin C (50 mg/kg, 100 mg/kg, and 400 mg/kg) also showed no difference in packed cell volume compared to the pentylenetetrazole kindled rats group treated with lamotrigine only. (Figure 3)

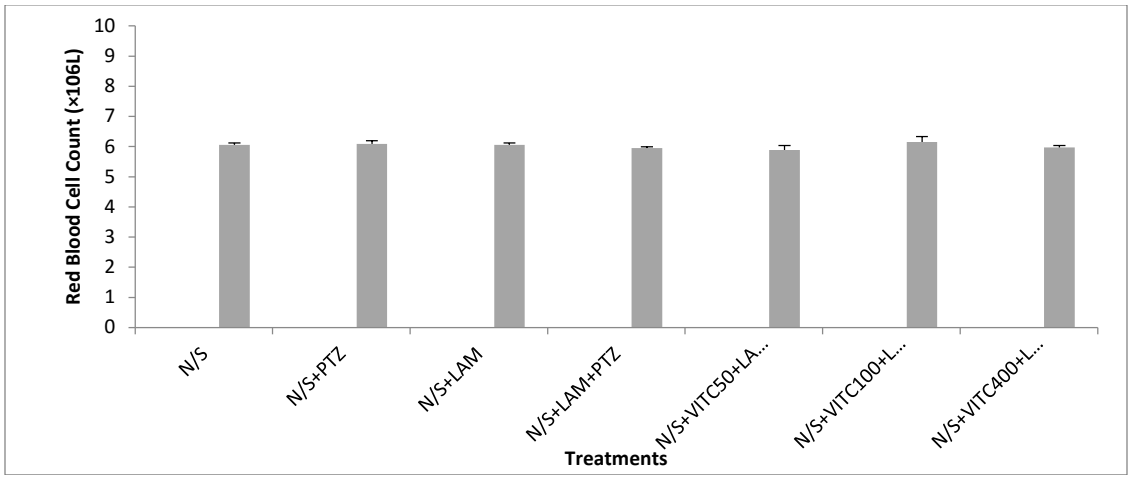


Pentylenetetrazole-induced Kindled Rats
Values are presented as Mean ± SEM. One way ANOVA, no statistically significant difference when test groups are compared against N/S control group. n = 8, PCV = Packed Cell Volume NS = Normal Saline, VITC = Vitamin C, PTZ = Pentylenetetrazole, LAM = Lamotrigine.

Fig 3: Effects of Vitamin C and Lamotrigine Co-administration on Packed Cell Volume in Pentylenetetrazole-induced Kindled Rats

Effects of Vitamin C and Lamotrigine Co-administration on Red Blood Cell Count in Pentylenetetrazole-induced Kindled Rats

There was no statistically significant difference in red blood cell count with vitamin C at all tested doses when compared to the control group. Red blood cell values are similar across all the groups. (Figure 4)

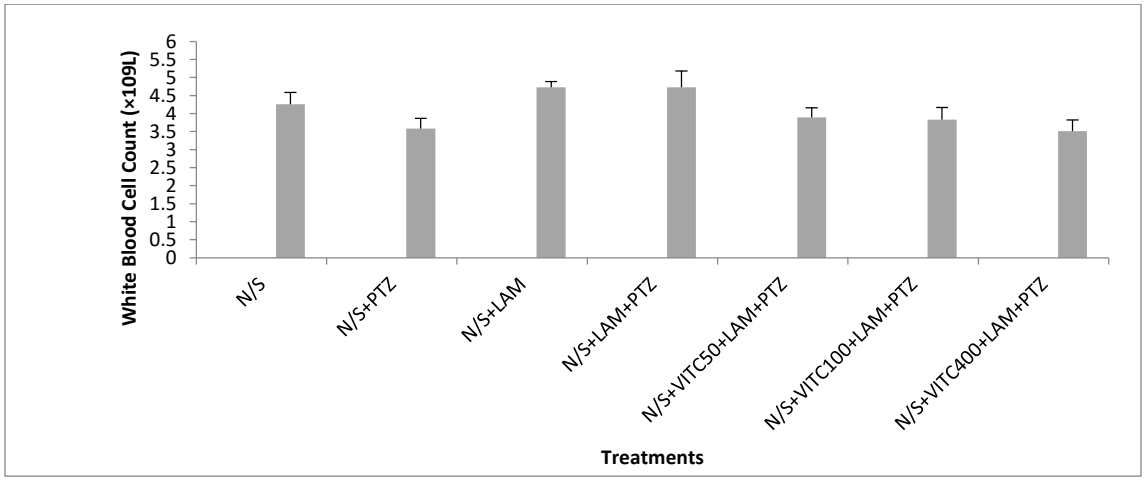


Values are presented as Mean ± SEM. One way ANOVA, no statistically significant difference when test groups are compared against N/S control group. n = 8, NS = Normal Saline, VIT C = Vitamin C, PTZ=Pentylenetetrazole, LAM=Lamotrigine.

Fig 4. Effects of Vitamin C and Lamotrigine Co-administration on Red Blood Cell Count in Pentylenetetrazole-induced Kindled Rats

Effects of Vitamin C and Lamotrigine Co-administration on White Blood Cell Count in Pentylenetetrazole-induced Kindled Rats

There was no statistically significant difference in white blood cell count in all treatment groups. (Figure 5)

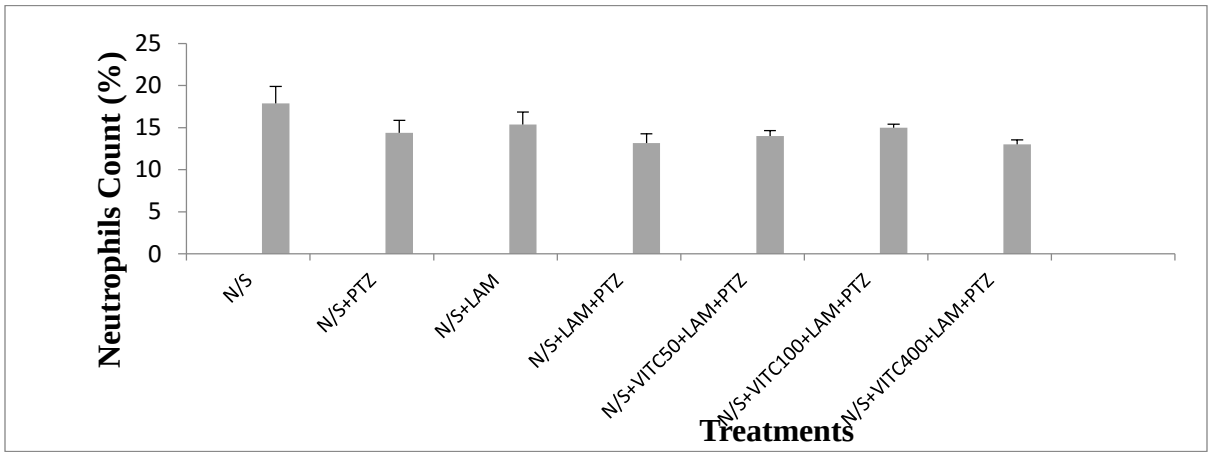


Values are presented as Mean ± SEM. One way ANOVA, no statistically significant difference when test groups are compared against N/S control group. n = 8, WBC = White Blood Cell Count NS = Normal Saline, VITC = Vitamin C, PTZ=Pentylenetetrazole,LAM=Lamotrigine.

Fig 5: Effects of Vitamin C and Lamotrigine Co-administration on White Blood Cell Count in Pentylenetetrazole-induced Kindled Rats

Effects of Vitamin C and Lamotrigine Co-administration on Neutrophil Count in Pentylenetetrazole-induced Kindled Rats

VitC400+LAM treated group showed a significant (^{a} $p< 0.05$) decrease in the number of neutrophils as against the control (N/S) group. (Figure 6)

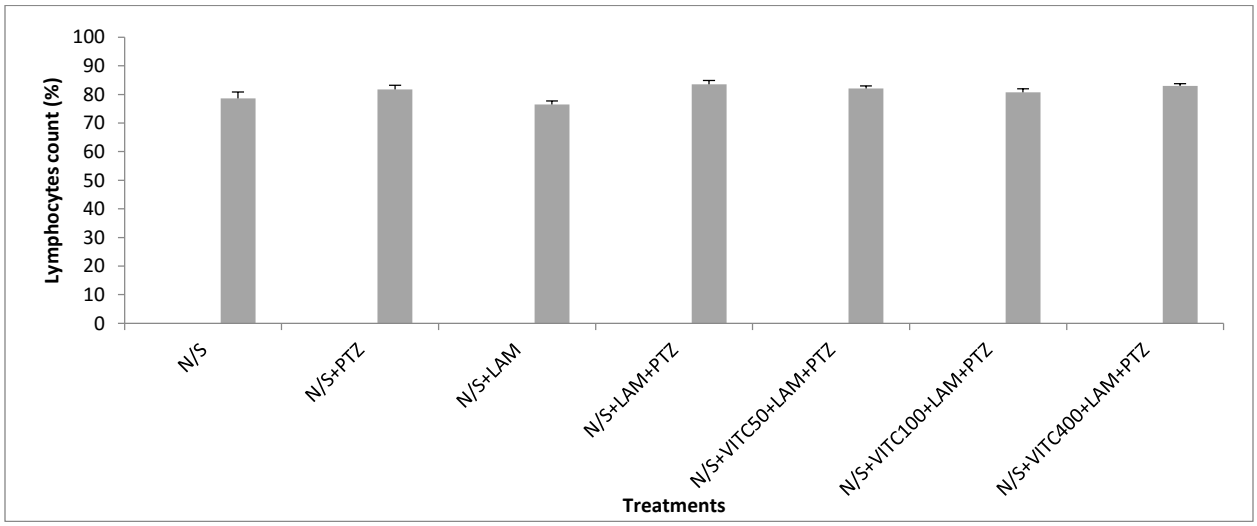


Values are presented as Mean ± SEM, a = $p< 0.05$ compared to normal saline control group. One way ANOVA followed by Dunnett's post hoc test. n = 8, NS = Normal Saline, VIT C = Vitamin C, PTZ=Pentylenetetrazole, LAM=Lamotrigine.

Fig. 4.6: Effects of Vitamin C and Lamotrigine Co-administration on Neutrophil Count in Pentylenetetrazole-induced Kindled Rats

Effects of Vitamin C and Lamotrigine Co-administration on Lymphocyte Count in Pentylenetetrazole-induced Kindled Rats

Vitamin C (400 mg/kg) showed a statistically significant increase in the number of lymphocytes as against the lamotrigine only treated group. (Figure 7)

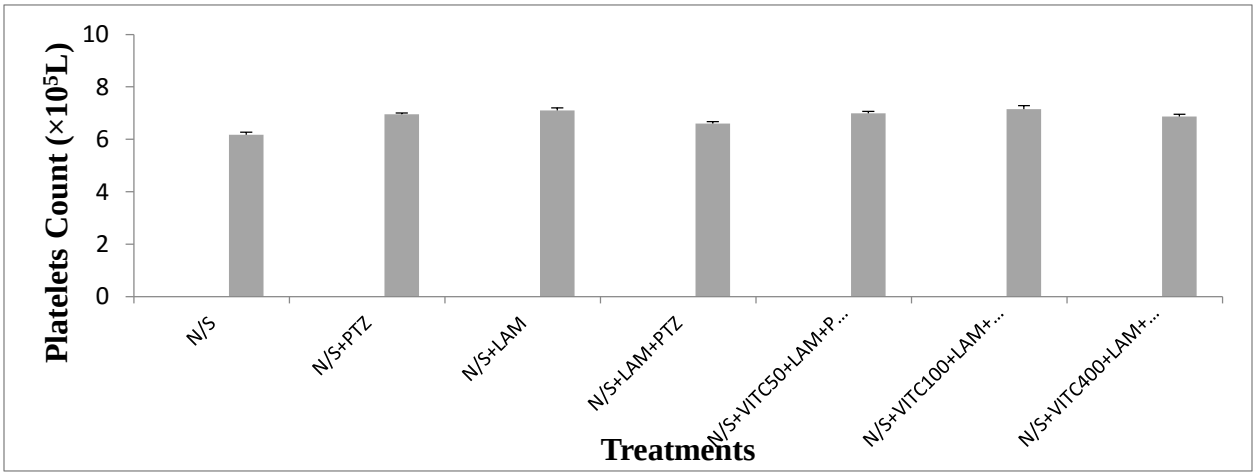


Values are presented as Mean ± SEM, One way ANOVA followed by Dunnett's posthoc test. n = 8, NS = Normal Saline, VIT C = Vitamin C, PTZ = Pentylenetetrazole, LAM = Lamotrigine. b = p< 0.05 for N/S+LAM control group, c = p< 0.05 for N/S+LAM+PTZ and N/S+VITC400+LAM+PTZ control groups respectively.

Fig. 7: Effects of Vitamin C and Lamotrigine Co-administration on Lymphocyte Count in Pentylenetetrazole-induced Kindled Rats

Effects of Vitamin C and Lamotrigine Co-administration on Platelet Count in Pentylenetetrazole-induced Kindled Rats

There was no difference in platelet count in all treatment groups. (Figure 8)



Values are presented as Mean ± SEM. One way ANOVA, no statistically significant difference when test groups are compared against N/S control group. n = 8, NS = Normal Saline, VIT C = Vitamin C, PTZ=Pentylenetetrazole, LAM=Lamotrigine.

Fig. 8: Effects of Vitamin C and Lamotrigine Co-administration on Platelet Count in Pentylenetetrazole-induced Kindled Rats

DISCUSSION

Inspite of the numerous existing approaches to the treatment of epilepsy, most antiepileptic drugs, beside certain and unquestionable benefits, have some disadvantages that necessitate the search for new method of treatment. Maximal electroshock test is a model for generalized tonic clonic and partial seizures. Vitamin C have been shown to have anticonvulsant properties by decreasing peroxidation of lipids most possibly by influencing and escalating action of antioxidant enzymes like superoxide dismutase and catalase in the adult rat hippocampus (Santos *et al*., 2008; Santos *et al*., 2009) and works as a neuromodulator (Tome´Ada *et al*., 2010). Vitamin C has also been proved to reduce injury in the hippocampus during seizure (Sultana *et al*., 2013). Therefore, the anticonvulsant activities i.e protection against seizure induced by electroshock stimuli and also decrease in the mean recovery time from convulsion observed with sub-protective dose of lamotrigine in combination with vitamin C in this study could possibly be attributed to these effects. Kindling is a well established model for abnormal plasticity leading to seizures and to epilepsy (Rivara *et al*., 2012). Several studies have established that progression of seizures in kindling is associated with decreased number of $GABA_A$ receptor binding sites in hippocampus (Bazyan *et al*., 2001), amplification of glutamate release and elevated nitric oxide level (Riazi *et al*., 2006). The chloride channel of the $GABA_A$ receptor is responsible for the rapid hyperpolarization of paroxysmal depolarizing state involved in kindling, leading to increase in seizure severity (Armijo *et al*., 2000). In a study conducted by (Gonza´lez-Ramı´rez *et al*., 2010), immature rats pretreated with vitamin C (500 mg/kg) for five days revealed a significant retardation of onset to myoclonic, clonic and tonic seizures or even the absence of convulsion (induced by pentylenetetrazole) was noted. Vitamin C also decreased the frequency of seizures and mortality in rats. Vitamin C (300mg/kg) was also shown to protect against seizures, to prolong seizure duration at (100 mg/kg) and to exert no effect on seizure at (30 mg/kg) (Schneider *et al*., 2004). Moreover, a reduction in amino acids (GABA, glutamate and aspartate) concentration following vitamin C supplementation, has been observed in many regions of brain, especially in hippocampus and amygdala (Gonza´lez-Ramı´rez *et al*., 2010). Findings in this

study revealed that lamotrigine alone as an anticonvulsant agent was able to reduce severity of the seizure by not allowing the seizure to reach last stage (i.e seizure score 5 at the end of the kindling experiment). Additionally, combination of lamotrigine and vitamin C at all doses tested caused a further decrease in seizure severity. The statistically significant reduction in seizure severity observed in this study with vitamin C at doses of 50 mg/kg and 400 mg/kg suggest a possible potentiation effect of vitamin C on lamotrigine anticonvulsant activity. These findings are similar to that earlier described by (Gonza´lez-Ramı´rez *et al*., 2010). Almost all classes of psychotropic agents have been reported to cause blood dyscriasis, and agranulocytosis is probably the most important drug-related dyscriasis (Young, 1994; Flanagan and Dunk, 2008). Idiosyncratic bone marrow suppression is a life-threatening event that is not related to dose or to the duration of administration and is not predicted by repeated blood draws (Sepkuty and Kaplan, 2004). Indeed idiosyncratic aplastic anemia is one of the adverse drug reaction associated with all major antiepileptic drugs except gabapentin (Scheuer, 1996). The use of lamotrigine have been associated with neutropenia which appeared 14 days after commencement of lamotrigine therapy (Kellie *et al*., 2005). Published reports of lamotrigine-induced neutropenia (de Camargo and Bode 1999; Damiani and Christensen 2000; Fernandez-Galan *et al*., 2000; Solvason, 2000; Fadul *et al*., 2002; Lambert *et al*., 2002; Norman *et al*., 2002; Le Drew *et al*., 2005) have demonstrated normalization of blood counts with drug discontinuation alone. The decrease in neutrophils observed in the lamotrigine only treated group is similar to the findings of (Damiani and Christensen, 2000; Lambert *et al*., 2002; Norman *et al*., 2002; Le Drew *et al*., 2005) who were able to demonstrate normalization of blood neutrophils count following drug discontinuation alone. An increase in neutrophils was observed across all the groups treated with a combination of vitamin C and lamotrigine. Again, there was a decrease in PCV in groups treated with lamotrigine. This finding is similar to that of (Cocito *et al*., 1994). Lamotrigine may have exerted these effects possibly by involving enzymatic inhibition of dihydrofolatereductase by lamotrigine. Increased PCV level was observed in groups treated with vitamin C and lamotrigine combination which is possibly due to the haemopoeitic effect of vitamin C (Sharon, 2013). Overall, haemoglobin concentration, white blood cell count, red blood cell and platelet counts were observed to be within normal limits across all treatment groups. These findings are similar to that of an earlier study (Piyush *et al*., 2007).

CONCLUSION

The outcome of the study provides evidence that vitamin C possesses significant effects on the anticonvulsant properties and some haematological side effects of lamotrigine in laboratory animals. The results thus suggest that vitamin C can serve as an adjunct in treatment of epilepsy using lamotrigine.

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