

*Full Length Research Paper*

## Effects of administration of carbamazepine and/or phenytoin on haematological parameters in wistar rats

Hadiza Aliyu<sup>1\*</sup>, Joseph O. Ayo<sup>2</sup>, Suleiman F. Ambali<sup>1,3</sup> and Abdulkadir U. Zezi<sup>4</sup>

<sup>1</sup>Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria.

<sup>2</sup>Department of Veterinary Physiology, Ahmadu Bello University, Zaria, Nigeria.

<sup>3</sup>Department of Physiology and Pharmacology, University of Ilorin, Ilorin, Nigeria.

<sup>4</sup>Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences Ahmadu Bello University, Zaria, Nigeria.

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The aim of the study was to evaluate the haematological alterations following the administration of carbamazepine (CBZ) and/or phenytoin (PHE). Forty apparently, healthy male adult Wistar rats weighing between 144 and 300 g were used for the experiment. They were divided into four groups of 10 animals each. Rats in group I (controls) were given distilled water at the dose of 2 ml/kg and they served as untreated controls. 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), respectively. All treatments were administered orally by gavage. The regimens were given once daily for a period of eight weeks. At the end of the experiment, the rats were sacrificed and blood samples were collected for the evaluation of total erythrocyte (RBC) count, packed cell volume (PCV), haemoglobin (Hb) concentration, and platelet counts. Also, total and differential leucocyte counts were evaluated using standard laboratory procedures. There was no significant ( $P > 0.05$ ) change in the value of the PCV, Hb and platelets but the RBC decreased ( $P < 0.01$ ) in the CBZ-treated group. There were increases in lymphocytes ( $P < 0.05$ ) and neutrophils ( $P < 0.01$ ) in rats treated with CBZ. In conclusion, the administration of CBZ caused alterations in haematological parameters, the changes observed in the other treatment groups which are PHE and CBZ + PHE are not statistically significant. Haematological parameters should be strictly monitored regularly in individuals administered with CBZ and/or PHE. If there are persistent alterations, the administration of the drugs should be discontinued.

**Key words:** Carbamazepine, phenytoin, rats, oral administration, 8 weeks, haematological parameters.

### INTRODUCTION

Epilepsy is one of the frequent neurological disorders (Ashrafi et al., 2010), encompassing a group of syndromes that vary in its associated pathology and seizure types (Nair, 2003). The characteristic event in epilepsy is the seizure, which is associated with the

episodic high frequency discharge of impulses by a group of neurones (Rang et al., 2005). Phenytoin, phenobarbitone and carbamazepine are the first-line antiepileptic drugs; these first-line drugs are commonly used because of their efficacy and low cost (Misra et al., 2003). Almost

\*Corresponding author. E-mail: haliyu63@gmail.com. Tel: +2348027149001.

all classes of psychotropic agents have been reported to cause blood dyscrasia, and agranulocytosis is probably the most important drug-related blood dyscrasia (Flanagan and Dunk, 2008). The rationale for combining some antiepileptic drugs (AEDs) is usually based on the presumptions concerning two aspects of efficacious treatment; the first is related to the anticonvulsant activity of the combining drugs, while the second takes into consideration the side-effects profile of the co-administered AEDs (Luszczki, 2004).

After the use of a first and a second antiepileptic drug without adequate improvement, a combination of two drugs is used (Kwan and Brodie, 2000). Successful treatment consists of finding the balance between obtaining adequate seizure control and avoiding adverse effects (Chung et al., 2005). Phenytoin (PHE) sodium is an anticonvulsant used to control 'grand mal' and psychomotor seizures (Vijay et al., 2009). Systemic administration induces anticonvulsant effect in humans and experimental animals (Ryakaczewska-Czerwińska, 2007). It exerts anti-seizure activity without causing general depression of the central nervous system, but the most significant effect of phenytoin is its ability to modify the pattern of maximal electroshock seizures (McNamara, 2006). Phenytoin blocks voltage-sensitive sodium ion channels and in this way inhibits neuronal firing in the brain (Ryakaczewska-Czerwińska, 2007). It alters potassium and calcium ion conductance, membrane potentials, concentrations of amino acids and the neurotransmitters - noradrenaline, acetylcholine and  $\gamma$ -aminobutyric acid (GABA) receptors. It paradoxically causes excitation in some cerebral neurones; a reduction of calcium permeability with inhibition of calcium influx across the membrane (Porter and Meldrum, 2007). It can cause gingival hyperplasia, agranulocytosis and aplastic anaemia deficits when given for a long time (Vijay et al., 2009).

Carbamazepine (CBZ) is a highly conventionally used antiepileptic drug, which has efficacy in attenuating picrotoxin-induced convulsion (Ali et al., 2003) and against maximal electroshock seizures (Porter and Meldrum, 2007). This may be attributed to its mechanism of action; that is, its use dependent sodium channel blockade, weak GABAergic and antigitamatergic effects (Motohashi, 1992). CBZ is the usual drug of choice for patients with newly diagnosed partial onset seizure (Gamble et al., 2009). The rate of absorption varies widely among patients, although almost complete absorption apparently occurs in all (Porter and Meldrum, 2007). Haematological toxicity of CBZ is well documented, and a patient undergoing CBZ therapy should be carefully monitored, especially for serious adverse reactions including pure red cell aplasia (Tagawa et al., 1997).

The aim of the study was to determine the haematological alterations that may accompany the use of these drugs in male adult Wistar rats.

## MATERIALS AND METHODS

### Animals

Forty adult male Wistar 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 Physiology and Pharmacology, Ahmadu Bello University, Zaria and were housed in rat cages. The animals were fed pellets made from grower's 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.

### Anticonvulsant drugs

The anticonvulsant drugs used in this study were carbamazepine (CBZ) tablets (Hovid Bhd, Malaysia) at 20 mg/kg and phenytoin (PHE) capsules (BIOMEDICINE Belgium) at 100 mg/kg.

### Experimental protocols

The animals were divided at random into four groups of 10 animals each. Animals in groups 2, 3 and 4 were given CBZ (20 mg/kg), PHE (100 mg/kg) and CBZ + PHE (20 and 100 mg/kg, separately), respectively. Rats in group 1 were given distilled water at 2 ml/kg and served as untreated controls. All treatments were administered orally by gavage once daily for a period of eight weeks.

### Evaluation of the effect of CBZ and/or PHE on haematological parameters

The haematological parameters of erythrocytic mass [packed cell volume (PCV), total red blood cell (RBC) count, haemoglobin (Hb), total and differential leucocyte counts and platelet count] were analysed using an auto-analyzer (ADVIA 60<sup>®</sup> Haematology System, Bayer Healthcare, Bayer Diagnostics Europe Ltd., Chapel Lane, Swords, Co., Dublin, Ireland; manufactured in France for Bayer) in the Haematology Laboratory, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

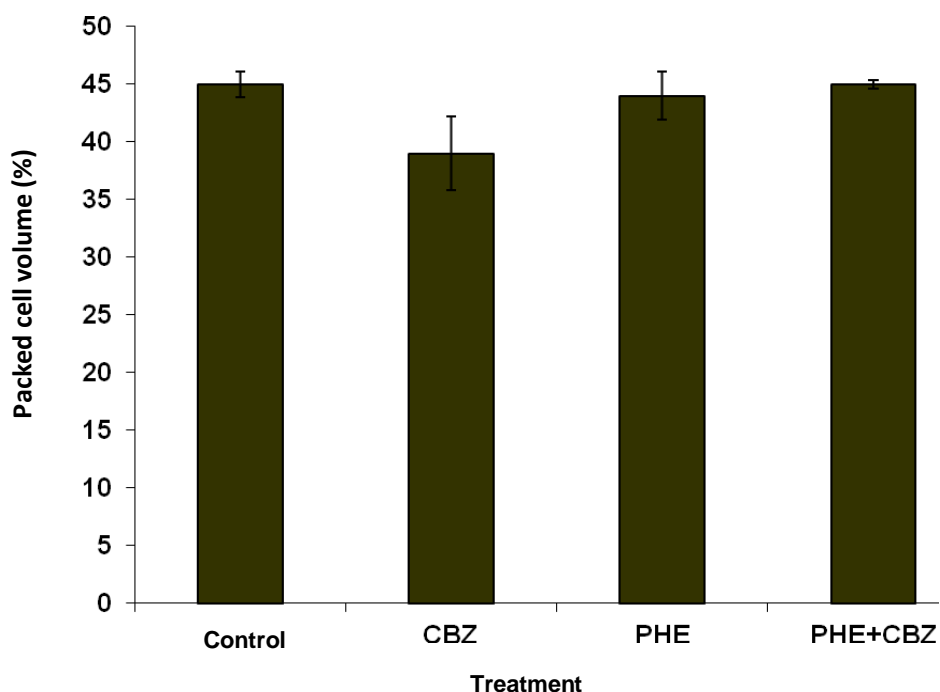
### Statistical analysis

Values obtained were expressed as mean  $\pm$  standard error of mean (SEM) and subjected to statistical analysis using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. The program used for the analysis was GraphPad Prism, Version 4.0 for Windows from GraphPad Software, San Diego, California, USA ([www.graphpad.com](http://www.graphpad.com)). Values of  $P < 0.05$  were considered significant.

## RESULTS

### Effect of treatments on the packed cell volume

There was no significant difference in the value of the PCV obtained in the CBZ, PHE and CBZ + PHE groups, compared to the control group. Also, there was no significant difference in PCV value between the drug-



**Figure 1.** Changes in the value of the PCV in the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/ka (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks was not statistically significant when compared with the control group.

treated groups (Figure 1).

#### Effect of treatments on red blood cell counts

The change in RBC counts obtained in the PHE and CBZ + PHE groups when respectively compared to that of the control group were not significant ( $P > 0.05$ ). The RBC counts in the CBZ group was lower ( $P < 0.01$ ) than the value recorded in the control group. There was no significant ( $P > 0.05$ ) change in RBC counts in between the drug-treated groups (Figure 2).

#### Effect of treatments on haemoglobin concentration

There was no significant ( $P > 0.05$ ) change in Hb concentrations between the experimental groups (Figure 3).

#### Effect of treatments on platelet counts in Wistar rats

There was no significant change in the platelet counts when the values obtained in the drug-treated groups were respectively compared to that of the control group. Also, the changes recorded in the counts between the drug-treated groups were not different ( $P > 0.05$ ) (Figure 4).

#### Effect of treatments on total leucocyte counts in Wistar rats

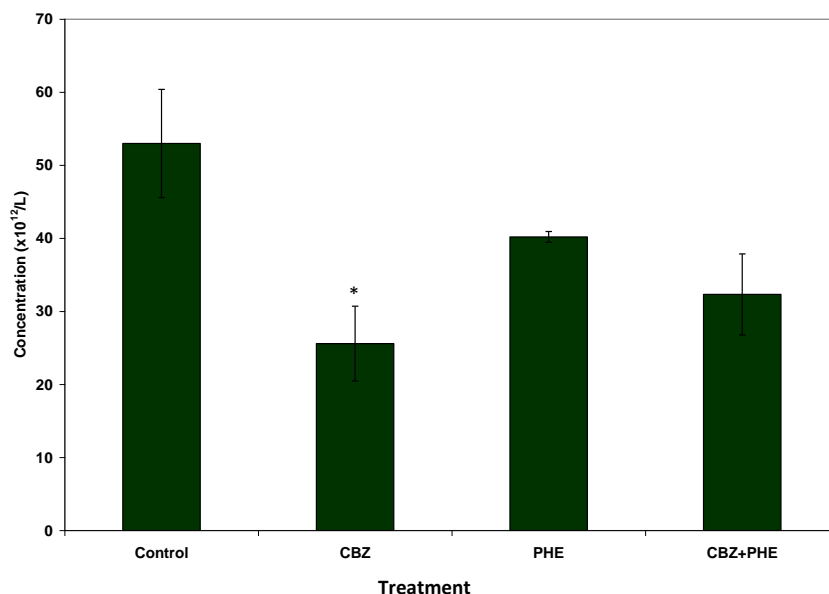
An insignificant increase was observed when the total leucocyte counts in each of the drug-treated group were compared to that of the control group. There was no significant increase in the total leucocyte counts obtained between the drug-treated groups (Figure 5).

#### Effect of treatments on neutrophil counts in Wistar rats

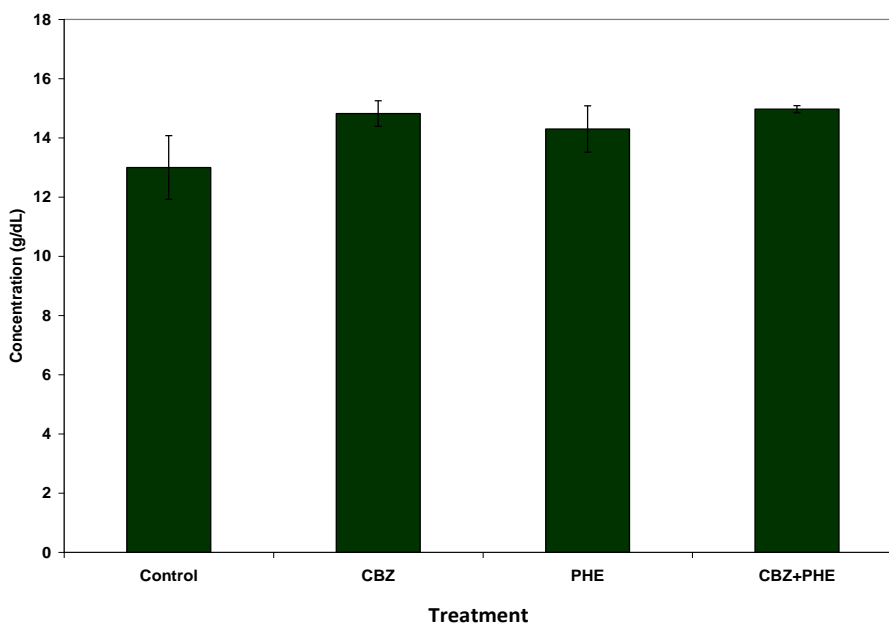
Neutrophil counts in the CBZ group rose significantly ( $P < 0.01$ ) when compared to that of the control group, but the increase in the PHE and CBZ + PHE groups was not different from that of the control group. There was no significant change in neutrophil counts obtained in between the treatment groups (Figure 6).

#### Effect of treatments on lymphocyte counts

The lymphocyte counts increased ( $P < 0.05$ ) in the CBZ group when compared to that of the control group. There was no significant ( $P > 0.05$ ) change in lymphocyte counts when the PHE and CBZ + PHE groups were



**Figure 2.** The value of RBC in the drug-treated groups decreased upon the administration of the drugs at a dose rate of 20 mg/kg (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks when compared with control group. \* =  $P < 0.01$  (CBZ versus control).

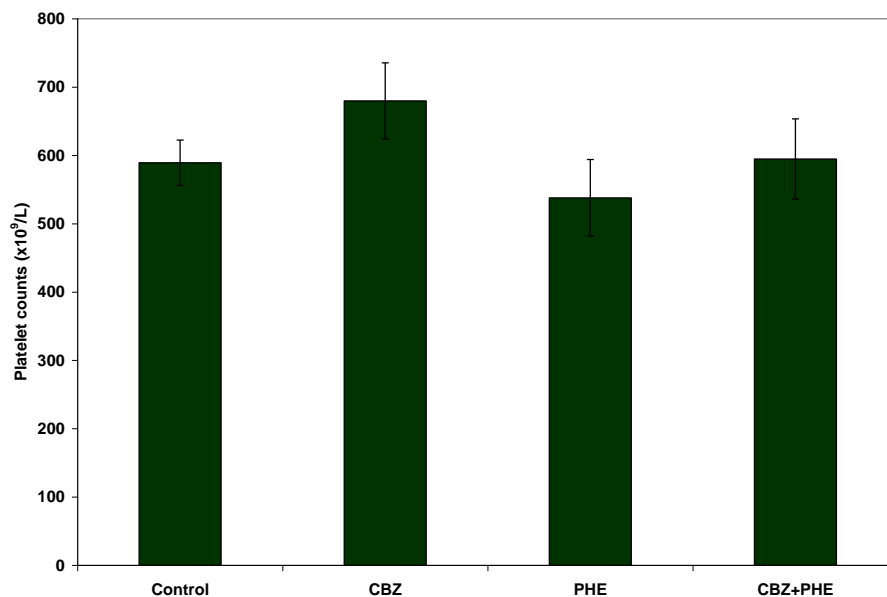


**Figure 3.** Changes in Hb concentration in the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/kg (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks were not statistically significant when compared with control group.

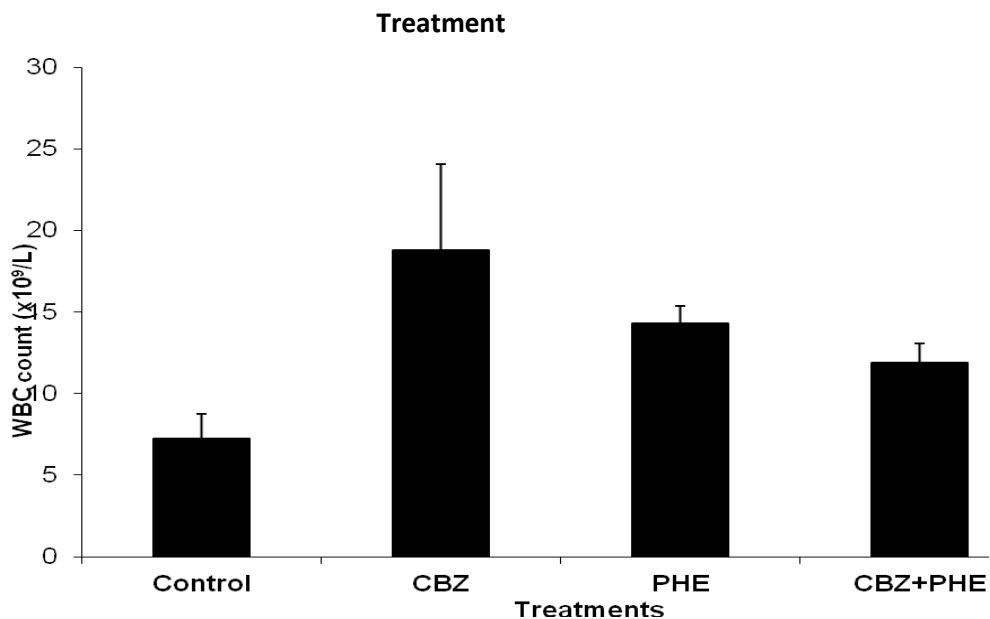
respectively compared to the control group. Similarly, no significant ( $P > 0.05$ ) changes in lymphocyte counts were recorded in between the drug-treated groups (Figure 6).

## DISCUSSION

The decrease in RBC count in the CBZ, PHE and CBZ +



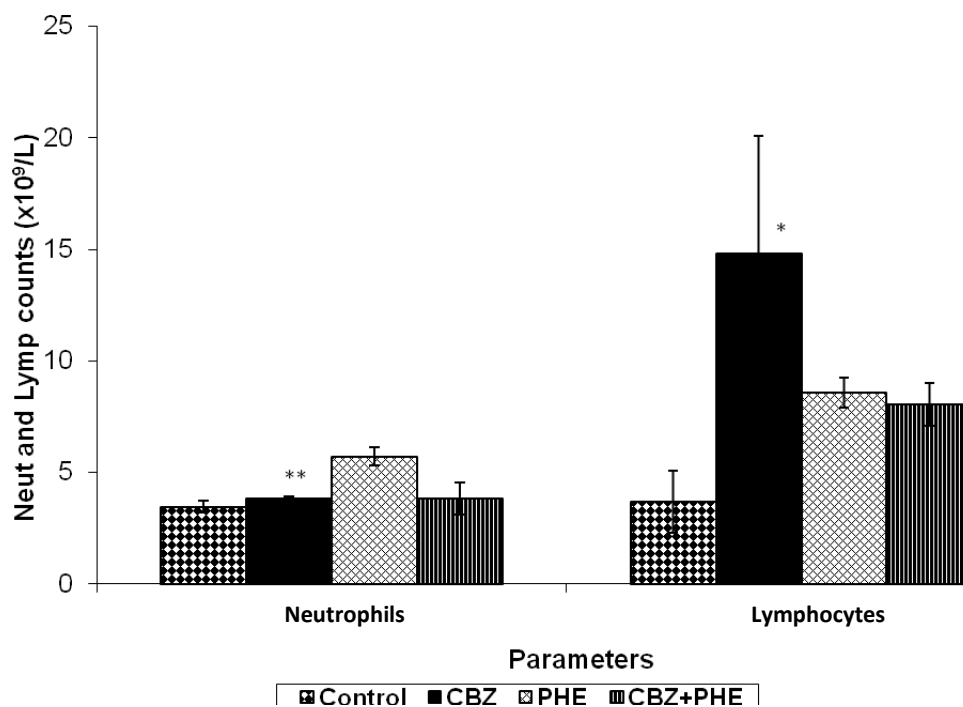
**Figure 4.** Changes in the platelet counts in the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/kg (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks were not statistically significant when compared with the control group.



**Figure 5.** Changes in the WBC counts in the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/kg (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks were not statistically significant when compared with the control group.

PHE groups agreed with the finding of Misra et al. (2003) who observed that PHE, phenobarbital and CBZ are highly toxic to the haemopoietic system. Thakur et al.

(2011) showed that decrease in RBC count in the PHE group may be due to the fact that the drug undergoes oxidative metabolism which resulted in the formation of a



**Figure 6.** The neutrophil and lymphocyte counts increased in the drug-treated groups upon the administration of the drugs at a dose rate of 20 mg/kg (CBZ) and 100 mg/kg (PHE) per os for a total period of 8 weeks when compared with the control group. \* =  $P < 0.05$  (CBZ versus control), \*\* =  $P < 0.01$  (CBZ versus control).

toxic arene oxide intermediate. This oxide covalently binds with cell macromolecules, causing cytotoxic damage, bone marrow toxicity and aplastic anaemia. The significant decrease in RBC count in the CBZ group was similar to the result obtained by Tagawa et al. (1997) who suggested that it may be due to isolated cessation of RBC production, resulting from pure RBC aplasia. However, McNamara (2006) reported that the prevalence of aplastic anaemia appears to be 1 in 200,000 patients treated with CBZ monotherapy. Therefore, the concern that aplastic anaemia may be a frequent complication of long-term CBZ therapy may remain controversial, despite the result obtained in the present study.

The non-significant decrease in RBC counts in the polytherapy group indicated a minimal effect of co-administration of the drugs on the RBCs. Drugs have been shown to cause idiosyncratic bone marrow suppression or dose-related suppression (Kaufman et al., 1996). Idiosyncratic bone marrow suppression is a life-threatening event that is not related to dose or to the duration of administration and cannot be predicted by repeated blood draws (Young, 1994; Sepkuty and Kaplan, 2004). Indeed idiosyncratic aplastic anaemia is one of the adverse drug reactions associated with all major AEDs, except gabapentine (Scheuer, 1996). The

AEDs that are primarily known to be associated with bone marrow suppression (although rare) are felbamate, CBZ, PHE, and valproate (Suchitra and Bussel, 2000).

The significant neutrophilia recorded in the CBZ-treated group may be due to cellular inflammation in the absence of an infection (Ekaidem et al., 2006). Significant increase in lymphocytes observed in the CBZ-treated group may be induced by the stimulation of formation of epoxides by the activity of cytochrome P<sub>450</sub>. The epoxides have been shown to bind covalently with macromolecules and act as haptens to stimulate immunologic actions, hence lymphocytosis (Gerson et al., 1983; Spielberg et al., 1986). Minimal effects observed with co-administration of CBZ and PHE on haematological parameters compared to the monotherapy groups may be due to the fact that CBZ can reduce the bioavailability of serum PHE (Lai et al., 1992).

## Conclusion

The administration of CBZ and/or PHE caused alterations in haematological parameters which should be strictly monitored regularly in individuals administered with CBZ and/or PHE. If there are persistent alterations, the

administration of the drugs should be discontinued.

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