EFFECT OF VITAMIN E AND RESVERATROL ON OXIDATIVE STRESS AND HAEMATOLOGICAL PARAMETERS OF CARBAMAZEPINE-INDUCED OXIDATIVE STRESS IN MALE WISTAR RATS

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN HUMAN PHYSIOLOGY

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JUNE, 2017

DECLARATION

I declare that the work in this dissertation entitled: "EFFECT OF VITAMIN E AND RESVERATROL ON OXIDATIVE STRESS AND HAEMATOLOGICAL PARAMETERS OF CARBAMAZEPINE-INDUCED OXIDATIVE STRESS IN MALE WISTAR RATS" has been carried out by me in the Department of Human Physiology, Faculty of Medicine, under the supervision of Dr. M.I.A. Saleh and Dr. A. AbdulWahab. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Munira ALIYU _____ _____

Signature **Date**

CERTIFICATION

This dissertation entitled: "EFFECT OF VITAMIN E AND RESVERATROL ON OXIDATIVE STRESS AND HAEMATOLOGICAL PARAMETERS OF CARBAMAZEPINE-INDUCED OXIDATIVE STRESS IN MALE WISTAR RATS" by Munira ALIYU, meets the regulations governing the award of the Degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to Almighty ALLAH, Whom if not because of His Mercy and Guidance, this work would not have come to completion.

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ABSTRACT

Carbamazepine (CBZ) is a drug used in the treatment of epilepsy and neuropathic pain. Carbamazepine induces oxidative stress and haematological toxicity. Resveratrol, known as 3,5,4′-trihydroxystilbene, is found in grapes and other plant products. Resveratrol effectively scavenges free radicals and other oxidants. Vitamin E a lipid soluble antioxidant present in all cellular membranes. The present study was designed to assess the effect of vitamin E and resveratrol on oxidative stress biomarkers and hematological parameters of carbamazepine-induced oxidative stress in male Wistar rats. Adult male Wistar rats (n = 35) were grouped into seven (7) of five (5) rats each: Group I (control) received distilled water; Group II received 2 ml/kg of corn oil; Group III received 10g/L of carboxymethylcellulose; Group IV received 50 mg/kg of carbamazepine; Group V received 50 mg/kg of carbamazepine and 200 mg/kg of Vitamin E; Group VI received 50 mg/kg of carbamazepine and 20 mg/kg of Resveratrol; Group VII received 50 mg/kg of carbamazepine and the co-administration of vitamin E at 200 mg/kg and Resveratrol at 20 mg/kg. Administration was done orally for forty five (45) days. At the end of the experiment, the animals were sacrificed and the blood samples and its serum were used for haematological and biochemical analyses, respectively. The result of the present study revealed that carbamazepine significantly ($p<0.01$) decreased the levels SOD, CAT and GPx to 2.04 ± 0.02, 46.60 ± 0.40, 42.20 ± 0.49 respectively in comparison to control group. Treatment with carbamazepine also significantly ($p<0.01$) decreased RBCs, PCV, PLT, and WBCs to 6.85 ± 0.28, 39.08 ± 1.66, 256.00 ± 12.20, 4.20 ± 0.21 respectively when compared to the control group. Whereas carbamazepine significantly ($p<0.01$) increased MDA levels to 1.42 ± 0.04 when compared to control group. Administration of vitamin E significantly ($p<0.01$) increased SOD, CAT and GPx, to 2.42 ± 0.05, 51.80 ± 0.49, 47.60 ± 0.40 respectively and there was reduction in MDA (1.02 ± 0.05) when compared to CBZ treated group. Administration of vitamin E significantly ($p<0.05$) increased PLT (323.20 ± 23.83), non-significant increase in PCV (42.40 ± 0.76) and WBCs (4.80 ± 0.41) in comparison to CBZ treated group. Administration of resveratrol significantly ($p<0.01$) increased SOD, CAT and GPx, to 2.48 ± 0.05, 53.20 ± 0.37, 48.80 ± 0.37, respectively and reduced MDA level to 0.98 ± 0.05 when compared to CBZ treated group. Administration of resveratrol significantly ($p<0.01$) increased RBC(8.14 ± 0.21), PCV(48.88 ± 1.43), PLT(338.20 ± 3.98) and WBC(5.32 ± 0.20) in comparison to CBZ-treated group. The co-administration of vitamin E (200mg/kg) and resveratrol (20mg/kg) revealed a significant ($p<0.01$) increase in SOD (2.52 ± 0.04), CAT ($54.00\pm0.31$and GPx (49.20 ± 0.66) and significant ($p<0.01$) reduction in MDA (0.98 ± 0.05) in comparison to CBZ-treated group. Co-administration of vitamin E and resveratrol significantly ($p<0.01$) increased RBC (8.53 ± 0.15), PCV (50.70 ± 0.86), PLT (361.80 ± 8.46) and WBC (5.90 ± 0.18) when compared to CBZ-treated group. The present results support that vitamin E and resveratrol or their combination ameliorated carbamazepine induced oxidative stress in male Wistar rats.

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LIST OF ABBREVIATIONS

AEDs	=	anti-epileptic drugs
ATP	=	adenosinetriphosphate
CAT	=	catalase
CBZ	=	carbamazepine
CMC	=	carboxy methyl cellulose
CO	=	corn oil
DNA	=	deoxyribonucleic acid
DRESS	=	drug reaction with eosinophilia and symptoms
DW	=	distilled water
Fe_{2+}	=	iron (ferrous)
FMLP	=	formylmethionyl leucyl phenyalanine
GABA	=	gamma-aminobutyric acid
GPx	=	glutathione peroxidase
GSH	=	glutathione
H_2O_2	=	hydrogen peroxide
H_2O	=	water

MDA	=	Malondialdehyde
O_2	=	oxygen molecule
$\cdot O_2$	=	superoxide radical
$\cdot OH$	=	hydroxyl radical
OH^-	=	hydroxyl ion
PCV	=	packed cell volume
PLT	=	platelet
RBC	=	red blood cells
RESV	=	resveratrol
RNA	=	ribonucleic acid
RNS	=	reactive nitrogen species
ROS	=	reactive oxygen species
SOD	=	superoxide dismutase

CHAPTER ONE

1.0 Introduction

1.1 Background of the study

Whenever a cell's internal environment is perturbed by diseases, toxins or nutritional imbalance, mitochondria diverts electron flow away from itself, forming reactive oxygen species (ROS) and reactive nitrogen species (RNS), thus lowering oxygen consumption. This "oxidative shielding" acts as a defence mechanism for either decreasing cellular uptake of toxic pathogens or chemicals from the environment, or to kill the cell by apoptosis and thus avoid the spreading to neighbouring cells. Therefore, ROS formation is a physiological response to stress (Basir *et al.*, 2005). The term "oxidative stress" has been used to define a state in which ROS and RNS reach excessive levels, either by excess production or insufficient removal.

Epilepsy is a chronic neurological disorder characterized by seizures (Chang and Lowenstein, 2003). Many people with epilepsy have more than one type of seizures and may have other symptoms of neurological problems as well. Several antiepileptic drugs (AEDs) have been developed, but only a few of them have become established. It has been estimated that the majority of epileptic patients are treated with only four drugs viz.: phenobarbital, phenytoin, carbamazepine (CBZ) and valproic acid (BIaler and White, 2010).

Carbamazepine (CBZ), sold under the trade name Tegretol, is the most frequently prescribed medication used primarily in the treatment of epilepsy and neuropathic pain, administered alone or in combination with other medications (Brodie *et al.*, 2011).

It is widely used in the treatment of epilepsy, neuralgia and bipolar affective disorders (Jokeit *et al.,* 2001; Silvana *et al.,* 2013). Despite the availability of newer antiepileptic drugs, the drug is commonly used because of its efficacy and low cost (Abbondazo *et al.,* 1995; DeVriese *et al.,* 1995). Carbamazepine- induced toxicity may manifest as granulomatous hepatitis, severe cholestasis and hepatocytic necrosis (Aycicek and Iscan, 2007). Carbamazepine is an aromatic antiepileptic drug (AAED) which induces hepatotoxicity due to a defective detoxification by the epoxide hydrolase and accumulation of arene oxides. Epileptic children under antiepileptic monotherapy with Carbamazepine showed an imbalance in the serum oxidant/antioxidant status and it was suggested that such effects are associated with the side effects of these drugs (Aycicek and Iscan, 2007). Chronic carbamazepine treatment significantly decreased the superoxide dismutase, catalase, reduced glutathione levels and significantly increased the liver lipid peroxidation (Maheswari *et al*., 2014).

Carbamazepine in increased doses causes obvious oxidative stress inhibiting all antioxidant enzymes activities and reduced glutathione content (Li *et al.,* 2010). Numerous studies have examined and found that carbamazepine induces oxidative stress, possibly via the formation of free radicals and reactive oxygen species (ROS). ROS are produced through oxidative metabolism and can impose damage on cellular macromolecules such as mitochondria, endoplasmic reticulum, finally leading to cell death (Dal-pizzol *et al.,* 2000). Carbamazepine treatment decreased the levels of superoxide dismutase, catalase, and glutathione peroxidase, whereas it increased lipid peroxidation (Thakur *et al.,* 2012). A study done by Imad (2012) revealed that carbamazepine causes oxidative stress as represented by elevated serum malondialdehyde (MDA) levels.

Carbamazepine is associated with hematological changes including leucopenia, decreased red blood cell count, decreased hemoglobin, aplastic anemia, agranulocytosis, pancytopenia, thrombocytopenia and megaloblastic anemia (Thakur *et al.,* 2012). It has also been reported that people treated with carbamazepine showed evidence of haematological alterations (Homez *et al.*, 2004).

Antioxidant supplementation has been shown to combat oxidative stress in runners (Kelkar *et al.,* 2008). Vitamin E is a lipid soluble antioxidant present in all cellular membranes protecting against lipid peroxidation. It functions as a chain-breaking antioxidant by preventing chain initiation and propagation of free radical reaction and lipid peroxidation in cellular membrane. In addition to its antioxidant function, vitamin E influences the cellular response to oxidative stress through modulation of signal-transduction pathway (Azzi *et al*., 1992). Vitamin E was observed to significantly reduce the levels of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and bilirubin, the markers of hepatotoxicity elevated by CBZ and increase the levels of albumin and total protein depleted by CBZ (Maheswari *et al*., 2015).

Resveratrol, also known as 3,5,4′-trihydroxystilbene, is a polyphenolic phytoalexin found in grape skin (Bielsalski, 2007). It is best known for being found in high concentrations in red wine, but is also present in small amount in other plant products (Bielsalski, 2007). It has been found to possess anti-inflammatory, anti-cancer, and antioxidant properties, as well as its ability to increase lifespan in mammals (Baur *et al.*, 2006).

Resveratrol is synthesized in response to environmental stressors endogenously that include water deprivation, ultraviolet radiation and especially fungal infection. Thus, the production of

resveratrol in plants can be considered to be part of the defense mechanism (Hurst *et al*., 2008). Resveratrol effectively scavenges (neutralizes) free radicals and other oxidants (Stojonovic *et al.,* 2001) and inhibits low density lipoprotein (LDL) oxidation (Frankel *et al*., 1993; Brito *et al*., 2009). Resveratrol's antioxidant efficacy has been demonstrated in traumatic brain injury (Sönmez *et al.,* 2007; Ates *et al.,* 2007), methotrexate-induced liver toxicity (Tunali-Akbay *et al.,* 2010; Dalaklioglu *et al.*,2013), cisplatin and gentamicin-induced nephrotoxicity, and doxorubicin-induced cardiotoxicity (Silan *et al.,* 2007;Amaral *et al.,* 2008; Tatlidede *et al.,* 2009).

1.2 Statement of Research Problem

The pathogenesis of many diseases involves free radical formation and mediated lipid peroxidation of biological membrane resulting from oxidative stress conditions which is an essential factor in the exacerbation and complications of many diseases (Ogugua and Ikejiaku , 2005). Reactive Oxygen Species are highly reactive molecules and can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions contributing to many pathological conditions e.g. neurological disorders (Fitzpatrick *et al*., 2009). Numerous studies have examined and found that carbamazepine induces oxidative stress, possibly via the formation of free radicals and reactive oxygen species (ROS) thereby decreasing the levels of superoxide dismutase, catalase, and glutathione peroxidase, whereas it increased lipid peroxidation (Thakur *et al.,* 2012). Oxidative stress significantly reduce total red blood cells, hemoglobin, total white blood cells including neutrophils, platelets and packed cell volume (Thakur *et al.,* 2012).

Hence, the need to evaluate the effect of substances like Vitamin E and Resveratrol and their co-administration in oxidative stress conditions, in an attempt to provide a more effective remedy to this condition.

1.3 Justification

In current clinical practice, a great deal of attention has been given to antioxidants consumption in reducing oxidative stress. Administration of two antioxidants has shown high effectiveness in combating oxidative stress in the body (Abdel-Naim *et al.*, 1999). Resveratrol is known to have a scavenger effect on reactive oxygen species and a stabilizing effect on damaged cell membrane (Stojonovic *et al.,* 2001). Co-administration of antioxidants showed an improvement in packed cell volume, hemoglobin concentration and red blood cell count in rats subjected to heat stress (Alhassan *et al.,* 2010). Hence, this study was designed to determine if Vitamin E and Resveratrol or co-administration of Vitamin E and Resveratrol could ameliorate carbamazepine-induced oxidative stress in male wistar rats.

1.4 General Aim and Specific Objectives

1.4.1 General aim

The aim of the present study was to determine the effect of vitamin E and resveratrol on oxidative stress biomarkers and hematological parameters of carbamazepine-induced oxidative stress in male Wistar rats.

1.4.2 Specific objectives

The specific objectives of this study are as follows;

1. To determine the effect of vitamin E on full blood count (Red Blood Cell, White Blood Cells, Platelets, Packed Cell Volume) and oxidative stress bio-makers (Super Oxide Dismutase, Catalase, Glutathione peroxidase and Malondialdehyde) of carbamazepine-induced oxidative stress in male wistar rats.
2. To determine the effect of resveratrol on full blood count (Red Blood Cell, White Blood Cell, Platelet, Packed Cell Volume) and oxidative stress bio-makers (Super Oxide Dismutase, Catalase, Glutathione peroxidase and Malondialdehyde) of carbamazepine induced oxidative stress in male wistar rats.
3. To determine the effect of co-administration of vitamin E and resveratrol on full blood count (Red Blood Cell, White Blood Cells, Platelets, Packed Cell Volume) and oxidative stress bio-makers (Superoxide Dismutase, Catalase, Glutathione peroxidase and Malondialdehyde) of carbamazepine induced oxidative stress in male Wistar rats.

1.5 Null hypothesis:

Vitamin E and Resveratrol does not have any effect on biomarkers of oxidative stress and haematological parameters in carbamazepine-induced oxidative stress in male wistar rats.

1.6 Alternate hypothesis:

Vitamin E and Resveratrol have effect on biomarkers of oxidative stress and haematological parameters in carbamazepine-induced oxidative stress in male wistar rats**.**

CHAPTER TWO

2.0 Literature Review

2.1 Vitamin E

2.1.1 Description of Vitamin E

Vitamin E is a group of eight fat-soluble compounds that include both tocopherols and tocotrienols. It is the principal lipid soluble chain-breaking antioxidant in mitochondria, microsomes, and lipoproteins making it a good candidate for investigation of its effects against diseases that involve reactive oxygen species (ROS) as a main component (Maheswari *et al.*, 2015).

2.1.2 History of Vitamin E

Vitamin E was first used as a therapeutic agent in 1938 by Widenbauer who used wheat germ oil supplement on 17 premature newborn infants suffering from growth failure (Azzi *et al.*, 1992), eleven out of which recovered and were able to resume normal growth rates. Later on, in 1948, Gyorge and Rose noted that rats receiving tocopherol supplements suffered from less hemolysis than those that did not receive tocopherol (Azzi *et al.*, 1992; Brion et al., 2003). This he did while conducting experiments on alloxan effects on rats. In 1949, Gerloczy administered all-rac-α-tocopheryl acetate to prevent and cure edema; oral administration showed a positive response and intramuscular administration did not show a response (Brion et al., 2003). This served as the gateway to curing the vitamin E deficiency caused by hemolytic anemia described during the 1960s as the early investigative work on the benefits of vitamin E supplementation (Brion et al., 2003).

2.1.3 Forms of Vitamin E

There are eight forms of vitamin E divided into two groups, four of which are tocopherols while the other four are tocotrienols, and are identified by prefixes alpha-, beta-, gamma-, and delta-. Natural tocopherols occur in the RRR-configuration only while the synthetic form contains eight different stereoisomers and is called *all-rac*-α-tocopherol (Rahangadale *et al.*, 2012).

2.1.3.1 α-Tocopherol

This is the most biologically active form of vitamin E and the second most common form of vitamin E in the North American diet. It is an important lipid-soluble antioxidant. It performs its functions as antioxidant in what is known by the glutathione peroxidase pathway and it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Rahangadale *et al.*, 2012). This would remove the free radical intermediates and prevent the oxidation reaction from continuing. The oxidized α-tocopheroxyl radicals produced in this process may be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol or ubiquinol. Other forms of vitamin E have their own unique properties; for example, gamma-tocopherol is a nucleophile that can react with electrophilic mutagens (Brigelius, 2009).

2.1.3.2 Tocotrienols

Compared with tocopherols, tocotrienols are sparsely studied. The current research direction is starting to give more prominence to the tocotrienols, as the lesser known but more potent antioxidants in the vitamin E family. Some studies have suggested that tocotrienols have specialized roles in protecting neurons from damage and cholesterol reduction by inhibiting the

activity of HMG-CoA reductase; delta-tocotrienol blocks processing of sterol regulatory element-binding proteins (SREBPs). Oral consumption of tocotrienols is also thought to protect against stroke-associated brain damage in vivo (Das et al., 2008).

2.1.4 Functions of Vitamin E

Antioxidant function; vitamin E acts as a peroxyl radical scavenger, preventing the propagation of free radicals in tissues, by reacting with them to form a tocopheryl radical which will then be oxidized by a hydrogen donor (such as Vitamin C) and thus return to its reduced state. As it is fat-soluble, it is incorporated into cell membranes, which protects them from oxidative damage (Maheswari *et al.*, 2015).

Enzymatic activity regulator; for instance, protein kinase C (PKC), which plays a role in smooth muscle growth, can be inhibited by α-tocopherol. α-Tocopherol has a stimulatory effect on the dephosphorylation enzyme, protein phosphatase 2A, which in turn, cleaves phosphate groups from PKC leading to its deactivation, bringing the smooth muscle growth to a halt (Schneider, 2005).

It also has an effect on gene expression. Macrophages rich in cholesterol are found in the atherogenetic tissue. Scavenger receptor CD36 is a class B scavenger receptor found to be up-regulated by oxidized low density lipoprotein (LDL) and binds it (Devaraj et al., 2001). Treatment with alpha tocopherol was found to down regulate the expression of the CD36 scavenger receptor gene and the scavenger receptor class A (SR-A) (Devaraj et al., 2001) and modulates expression of the connective tissue growth factor (CTGF). CTGF gene, when

expressed, is responsible for the repair of wounds and regeneration of the extracellular tissue that is lost or damaged during atherosclerosis (Villacorta et al.,2003).

Vitamin E also plays a role in neurological functions, and inhibition of platelet aggregation. Its supplementation in cancer patients showed that it has an important neuroprotective effect as it has the ability to protect neuronal tissue in several neurodegenerative disorders including Alzheimer's disease (Muller, 2010).

2.1.5 Dietary sources of Vitamin E

Some of the cheap food items with vitamin E content include Wheat germ oil, Sunflower oil, Nuts and nut oils (like almonds and hazelnuts), Palm oil, leafy vegetables, Sweet potato, Tomatoes, Mangoes, lettuce, Rockfish among many others (Abner *et al.*, 2011).

2.1.6 Recommended daily intake

One IU (International Unit) of vitamin E is defined as equivalent to either: 0.67 mg of the natural form, RRR-alpha-tocopherol, also known as d-alpha-tocopherol; or 0.45 mg of the synthetic form, all-rac-alpha-tocopherol also known as dl-alpha-tocopherol (Institute of Medicine. Food and Nutrition Board, 2000).

Table 2.1: The Food and Nutrition Board at the Institute of Medicine (IOM) of the U.S.National Academy of Sciences (2000):

Developmental Stage	Age	Dose
Infants	0 to 6 months	4 mg/day
Infants	7 to 12 months	5 mg/day
Children	1 to 3 years	6 mg/day
Children	4 to 8 years	7 mg/day
Children	9 to 13 years	11 mg/day
Adolescents and Adults	14 and above years	15 mg/day

2.1.7 Health effects of Vitamin E

Vitamin E is a potent antioxidant with anti-inflammatory properties. Several lines of evidence suggest that among different forms of vitamin E, alpha-tocopherol (AT) has potential beneficial effects with regard to cardiovascular disease. AT supplementation in human subjects and animal models has been shown to decrease lipid peroxidation, superoxide production by impairing the assembly of nicotinamide adenine dinucleotide phosphate (reduced form) oxidase (Singh, *et al.*, 2005). vitamin E supplementation has various beneficial effects on the host immune system. The decreased cellular immunity with aging or during the development of AIDS is markedly improved by the intake of a high vitamin E diet. In addition, vitamin E plays an important role in the differentiation of immature T cells in thymus (Moriquchi and Muraqa, 2000).

While it was initially hoped that vitamin E supplementation would have a positive effect on health, research has not supported these conclusions. Vitamin E does not decrease mortality in adults, even at large doses, and may slightly increase it (Abner et al., 2011). It does not improve blood sugar control in an unselected group of people with diabetes mellitus or decrease the risk of stroke. Daily supplementation of vitamin E does not decrease the risk of prostate cancer and may even increase it (Bin et al., 2011). Studies on its role in age related macular degeneration are still ongoing as, even though it is of a combination of dietary antioxidants used to treat the condition. A Japanese study found that vitamin E may contribute to osteoporosis (Olson *et al.*, 2011).

2.1.8 Effects of Vitamin E Deficiency

Deficiency of Vitamin E can bring about the development of or cause:

- spinocerebellar ataxia
- myopathies
- peripheral neuropathy
- ataxia
- skeletal myopathy
- retinopathy
- impairment of the immune response
- Erythrocyte hemolysis (Kowdley et al., 1992).

2.2 Resveratrol

2.2.1 Description of Resveratrol

Resveratrol (3, 5, 4'–trihydroxystilbene) is a phytoalexin, a low-molecular weight secondary metabolite with antimicrobial activity – and a polyphenolic compound that belongs to the stilbene family. Chemically, resveratrol ($C_{14}H_{12}O_3$) is a white powder with a slight yellow cast having a molecular weight of 228 g/mol and a melting point of 253 - 255 ºC (Gehma *et al.*, 2004). It can be found in free (aglycone) or glycosylated form (piceid) and its oxidative dimerization leads to the formation of its polymer, the viniferins (Margarida and Afonso, 2013). Both aglycone and piceid exists in *cis* (Z)- or *trans* (E)- isomeric forms, because their two phenol rings (linked by a styrene double bond) generate the more stable form, *trans*-resveratrol; but, by UV photoisomerization, *trans*-resveratrol is converted to *cis*-resveratrol that has maximum absorbance at 286 nm, whereas the maximum absorbance for the *trans* isomeris achieved at 306

nm (Margarida and Afonso, 2013). Both isomers of resveratrol are extremely light sensitive and when protected from light, *trans*-resveratrol is stable for at least 28 days in buffers with pH ranging from 1 to 7, while *cis*-resveratrol is degraded at pH 10.0 (Mahdi *et al.*, 2014).

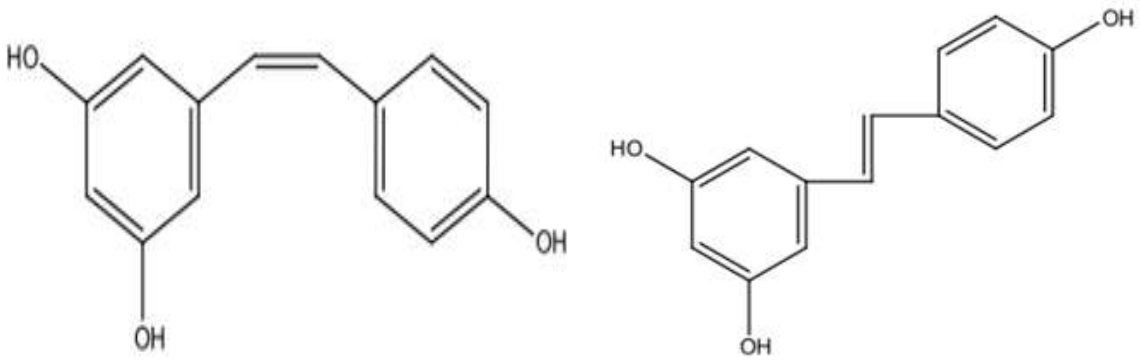

Figure 2.0: *cis*-resveratrol and *trans*-resveratrol (Margarida and Afonso, 2013).

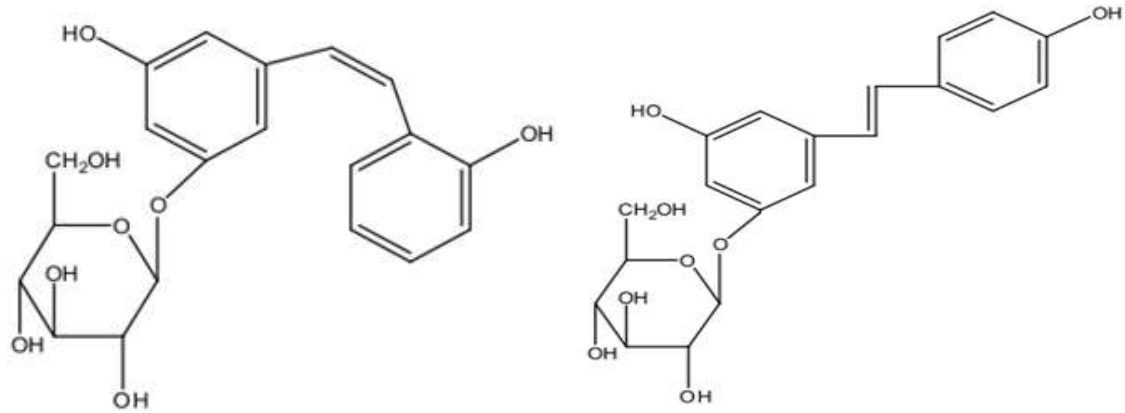

Figure 2.1: cis-piceid and trans-piceid (Margarida and Afonso, 2013).

2.2.2 Sources of Resveratrol

Resveratrol was first isolated from white hellebore (*Veratrum grandiflorum O. Loes*) by M. J. Takaoka in 1940, later in 1963. It was found in Itadori plant and *trans*-resveratrol was discovered in *Vitis vinifera* (grape vine) in 1976 by P. Langcake and R. J. Pryce (Mahdi *et al.*, 2014).

Resveratrol is found in abundant amount in red wine, grape berry skins and seeds and, particularly in dried roots of plant Polygonum cuspidatum (Hu *et al.,* 2013). Content of resveratrol in grapes varies from 0.16 to 3.54 mg/g, dry grape skin contains about 24 mg/g of resveratrol (Mukherjee *et al.,* 2010). Resveratrol is also present in other berries and nuts. For example cranberry raw juice contains about 0.2 mg/L. In other natural foods concentration of resveratrol varies in the range of mg/g (peanuts, pistachios) to ng/g (bilberries, blueberries) (Mukherjee *et al.,* 2010). It has been documented that red wine contains much greater amount of polyphenolic com- pounds than white wine. The concentration of resveratrol ranges from 0.1 to 14.3 mg/L in various types of red wine, while white wines contain only about 0.1–2.1 mg/L of resveratrol (Mukherjee *et al.,* 2010). Resveratrol can also be found in ferns, pines, legumes, white hellebore, pistachios, also in flowers and leaves such as eucalyptus, spruce, butterfly orchid and rheum (Sreenivasulu and Vijayalakshmi, 2010).

2.2.3 Biological activities of Resveratrol

After many tests to phytoalexins, there are numerous evidences for the clinical usefulness of resveratrol due to its wide range of biological activities via various mechanisms of action and targets. Between the broad applications of resveratrol, the major biological activities are antimicrobial and antioxidant activities, cardio and neuroprotective, cancer chemoprotective, prevention of ageing, reduction of obesity and inflammation, among other health beneficial effects of this phytoallexin (Das, 2011).

2.2.4 Production of Resveratrol

Resveratrol is commonly extracted/purified mainly from grapevine or chemically synthesized. However, in order to meet the market demand, it is necessary to accomplish alternative ways and/or improving existing pathways to produce this stilbene. Resveratrol oligomers ranging from dimers to octamers were found in nature and several dimers were successfully synthesized, some of the resveratrol dimers bearing a cyclopentane moiety include namely parthenocissin A (**Par**, **2**), quadrangularin A (**Qua**, **3**) and pallidol (**Pal**, **4**) (Li *et al.*, 2013).

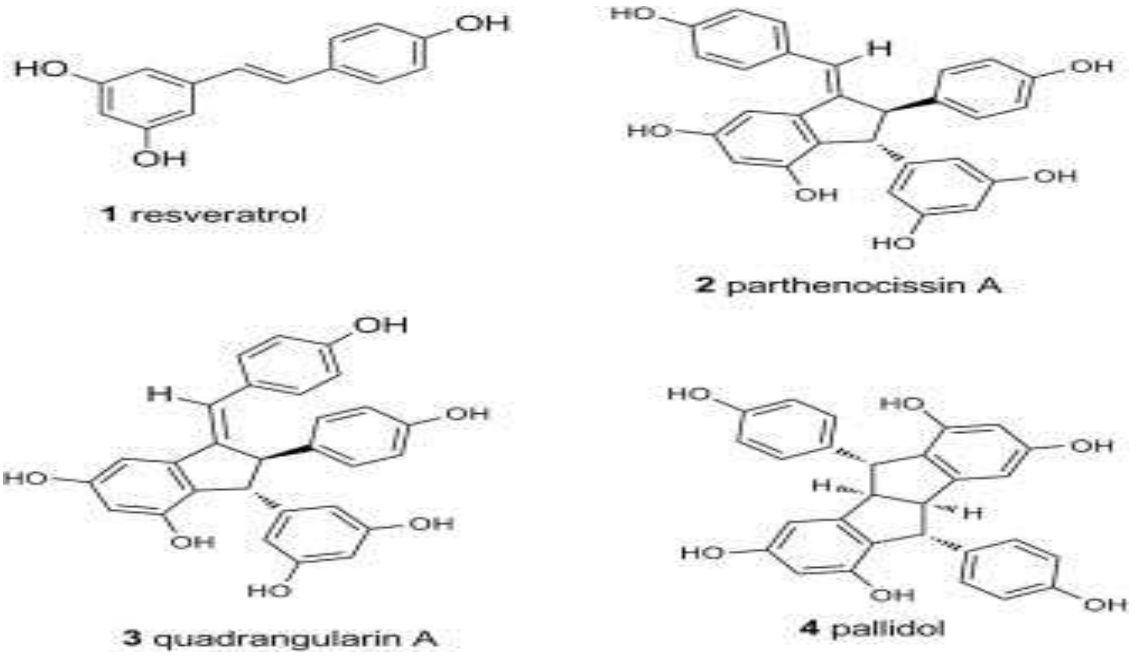

Figure 2.2: The structures of resveratrol monomer (**1**) and its three dimers, parthenocissin A (**2**), quadrangularin A (**3**) and pallidol (**4**) (Li *et al.*, 2013).

These three resveratrol dimers are among the most well-known oligostilbenoids that are biologically generated from two monomers in the presence of oxidases or peroxidases. These compounds have been shown as well to possess antifungal activity, fatty acid synthase (FAS) inhibitory activity, iNOS expression activity and weak antiproliferative activity (Silva *et al.*, 2012).

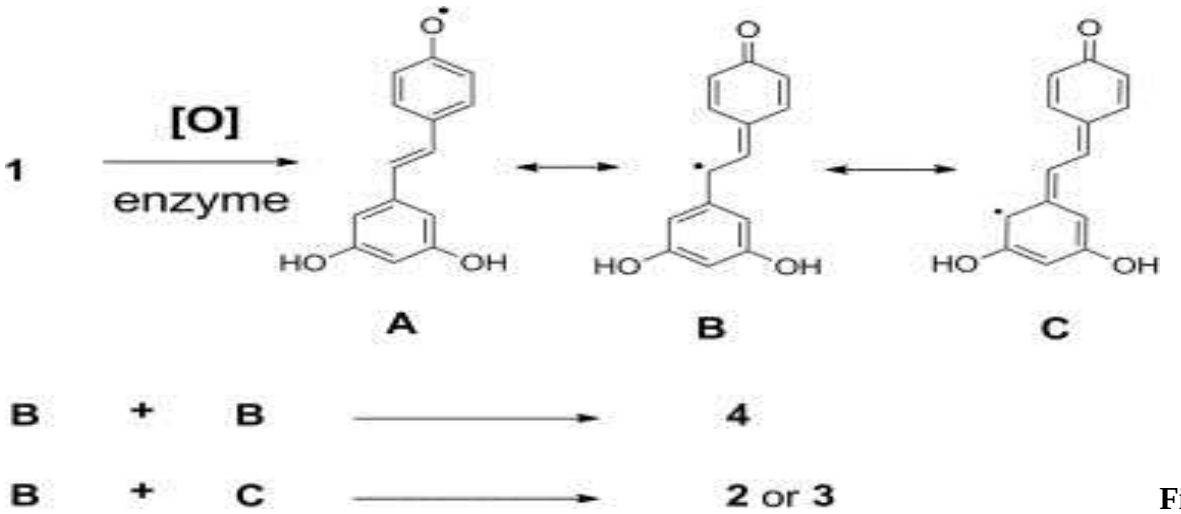

Figure 2.3: Biosynthetic pathway from **Res** to **Par**, **Qua** and **Pal** (Silva *et al.*, 2012).

2.2.5 Biosynthetic pathways of Resveratrol

Resveratrol as a plant secondary metabolite derived from shikimate-phenylpropanoid and/or polyketide pathway. The plant shikimate pathway has two end-products that are the entry to the biosynthesis of phenylpropanoids: phenylalanine and tyrosine. Resveratrol is formed on the phenylalanine/polymalonate pathway, being the last step of this biosynthetic pathway, and can be synthesized either from phenylalanine or tyrosine. Both phenylalanine and tyrosine precursors produce *para*-coumaric acid (*p*-coumaric acid, also known as *para*-hydroxycinnamic acid) (Surajit and Ka-Yun, 2011). Para-Coumaric acid is generated from phenylalanine through phenylalanine ammonia lyase (PAL) and cinnamate 4 hydroxylase (C4H), which acts in the cinnamic acid intermediate. Tyrosine ammonia lyase (TAL) exerts its activity directly in tyrosine. Then, *para*-coumaric acid is activated by ligation to coenzyme A (CoA) by 4-coumaroyl CoA ligase (4CL) and in the pathway-committing step, stilbene synthase (STS) condenses three units of malonyl-CoA (from fatty acids biosynthesis) with *para*-coumaroyl-CoA, forming a linear tetraketide molecule before a cyclization reaction carried out by STS, generating resveratrol. *trans*-Resveratrol can be modified to *trans*-piceid by 3-O-

glucosyltransferase (3-O-GT). However, resveratrol has several alternatives to its chemical biosynthesis, mainly its production by plant cell cultures and using recombinant microorganisms (Rocha-González *et al.*, 2008).

2.2.6 Resveratrol as a potent antioxidant

The effect of resveratrol depends on its redox status, i.e., it acts as an antioxidant or a prooxidant. The concentration of resveratrol and cell type are also important (De la Lastra and Villegas, 2007). Resveratrol is reported to be one of the most potent antioxidant against ROS and oxidative stress. ROS production by polymorphonuclear leukocytes stimulated by formylmethionyl leucyl phenyalanine (fMLP) can be strongly inhibited by resveratrol (Rotondo *et al.*, 1998).

Mizutani *et al.*, (2001) showed that resveratrol significantly reduces markers of oxidative stress like glycated albumin in serum and 8-hydroxyguanosine in urine in stroke-prone spontaneously hypersensitive rats. Moreover, resveratrol could act on blood cells and in lipoproteins. Resveratrol was incorporated into blood cells and lipoproteins after *in vitro* incubation with plasma, lipoproteins, and cells (Blache *et al.*, 1997). In fact, due to its lipophilic character, resveratrol is able to bind the lipoprotein particles suggesting that this event improved its antioxidant activity (Belguendouz *et al.*, 1998). The antioxidant function of resveratrol has been attributed to the blockade of radical propagation by formation of a resonance-stabilized peroxy radical (Kovacic and Somanathan, 2010). Resveratrol scavenges reactiveoxygen species (ROS) and reactive nitrogen species (RNS), promotes nitric oxide production, inhibits platelet aggregation, increases high-density lipoprotein cholesterol, inhibits lipid peroxidation and forms

protein carbonyls and increases antioxidant enzymes, such as superoxide dismutases (SODs), cata-lase and glutathione peroxidase (GPX) in a number of *in vitro* and *in vivo* systems (De la Lastra and Villegas, 2007; Gülc, 2010; Gambini, *et al.,* 2015).

Resveratrol was found to increase the level of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and its nuclear translocation which in turn activated antioxidant respon-sive elements (ARE) (Rubiolo *et al.,* 2008).

A pro-oxidant effect of resveratrol has been demonstrated in some studies. Dudley *et al.,* (2009) investigated how myocardial infarct size and cardiomyocyte apoptosis were affected in vivo by low and high doses of resveratrol; they found that cardioprotective properties of resveratrol were dose-dependent because at lower concentration (5 mM–10 mM) resveratrol functions as antioxidant, while at higher concentration it acts as pro-oxidant. Resveratrol may undergo autooxidation to produce super-oxide radical ($O2^{\bullet-}$), hydrogen peroxide ($H2O2$), and a complex mixture of semiquinones and quinones, which can become cyto-toxic (Sang *et al.,* 2007; Klaus *et al.,* 2010). It can act as a pro-oxidant in the presence of copper ions (Azmi *et al.,* 2005; Ahmad *et al.,* 2015).

2.2.7 Metabolism and bioavailability of Resveratrol

After oral intake in experimental animal models, about half of the resveratrol was found to be well absorbed quite rapidly whether as aglycone or on its glycosidic form from the digestive tract, especially in small intestine particularly via multidrug resistance-associated proteins 2 and 3 with extensive sulphate conjugation in humans (Ahmad *et al.,* 2015). The binding of the

compound to plasma proteins seems to occur particularly on albumin. The plasma half-life of free resveratrol ranges from 2 to 14 hours in humans, whereas in rats it has an average value of 1.4 hours (Markus and Morris, 2008).

2.2.8 Excretion of Resveratrol

One of the major ways for resveratrol elimination is renal excretion, resulting in high levels of resveratrol conjugates in urine, Due to small amount of resveratrol found in feces, fecal route was thought to be a minor route of elimination. However, approximately 50–80% of the compound is excreted in urine and feces and only 0.04% of free resveratrol is excreted in the urine within 24 h post-administration (Rocha-González *et al.*, 2008).

2.2.9 Toxicity of Resveratrol

Studies have shown that low doses of resveratrol may have a protective effect in human health, while high doses of this compound may be harmful. Nevertheless, data obtained from animal trials are promising and indicate the need for further human clinical trials in order to evaluate resveratrol effectiveness and toxicity towards a safe administration of this compound (Mahdi *et al.*, 2014). It was reported that resveratrol has low toxicity as it was well tolerated in the short-term experiments performed in humans (Almeida *et al.*, 2009; La porte *et al.*, 2010; Wong *et al.*, 2011). Recent clinical trials proved that resveratrol is well-tolerated and pharmacologically safe at doses up to 5 g/day (Patel *et al.*, 2011). Tome-Carneiro *et al.*,(2012) found that resveratrol treatment at low dose (8 mg/day) for one year significantly reduced a number of cardiac risk factors.

2.3 Epilepsy

2.3.1 Introduction of Epilepsy

Epilepsy is one of the most common neurological disorders, affecting about 50 million people worldwide (Maheswari *et al.*, 2015). It has been estimated that an approximate number of about 100 million people will have at least one epileptic seizure during their lifetime (Ambrósio *et al*., 2002). It encompasses a group of syndromes that vary in its associated pathology and seizure types. It may be associated with enhanced excitatory amino acid transmission, impaired inhibitory transmission or abnormal electrical properties of the affected cells (Maheswari *et al.*, 2015). The characteristic event in epilepsy is the seizure, which is associated with the episodic high frequency discharge of impulses by a group of neurons. It causes serious physical, psychological, social, and economic consequences. The estimated median prevalence of lifetime epilepsy for the developed countries is about 5.8 per 1,000 people and 10.3 per 1,000 people for developing countries (Rocha-González *et al.*, 2008).

2.3.2 Classification and etiology of Epilepsy

Epilepsy could be classified based on the severity of seizures into petit mal, focal or grand mal. In petit mal epilepsy, the seizure is usually very transient and is characterized by dizzy spells and a short lasting phase of syncope. Focal epilepsy shows characteristically certain parts of the body to be involved in the seizures. In grand mal epilepsy (status epilepticus), the individual goes into frank catatonic seizures being thrown down and accompanied by a phase of unconsciousness (Maheswari *et al.*, 2015).

Epilepsy could also be classified as idiopathic, provoked or symptomatic. Symptomatic epilepsies may have several causes that include trauma, tumor, infection, malformation or a systemic genetic disease; provoked seizures are predominantly caused by specific environmental or systemic factors and there are no significant neuroanatomical or neuropathological anomalies (Rocha-González *et al.*, 2008). Idiopathic epilepsy is defined as having a predominantly or presumably genetic cause and there are no significant neuroanatomical or neuropathological anomalies. Symptomatic epilepsy in young adults is usually the result of trauma during birth, congenital malformations or brain development anomalies, encephalitis, head trauma or a brain tumor (Shorvon, 2011).

2.3.3 Antiepileptic drugs (AEDs)

Phenytoin (PHE), carbamazepine (CBZ) and phenobarbitone are the first-line antiepileptic drugs commonly used because of their efficacy and low cost. CBZ is an anticonvulsant used to treat epilepsy and mood disorders. But it has been estimated that the majority of epileptic patients are treated with only four drugs viz.: phenobarbital, phenytoin, carbamazepine (CBZ) and valproic acid (BIaler and White, 2010).

Carbamazepine is the most frequently prescribed drug for the treatment of several forms of epilepsy and is administered alone or in combination with other medications to treat certain types of seizures in patients with epilepsy. Its main function is reduction of sustained repetitive firing in neurones by blocking voltage-gated sodium channels. CBZ exerts its therapeutic effects through the inhibition of brain neuronal activities. It is used for the treatment of seizure disorders and trigeminal and other neuralgias (Brodie *et al.*, 2011). Three principal pharmacological

targets of currently available AEDs as their mechanism of action are recognised: modulation of voltage-gated ion channels; enhancement of gamma-aminobutyric acid (GABA)-mediated inhibitory neuro-transmission; and attenuation of glutamate-mediated excitatory neurotransmission. However, there is need to improve understanding of the basic mechanisms of epilepsy, as the mechanisms involved in ictogenesis- that is initiation, amplification, and propagation of seizures-, differ from those involved in epileptogenesis (Rogawski, 2013).

2.3.4 Epilepsy and antioxidants

Induced seizures may be partially prevented with treatment using antioxidant substances, such as SOD mimetics, melatonin, Vitamins A, C. and E (Maheswari *et al.*, 2015). Investigation on the role of RNA oxidation in epileptogenesis using pilocarpine to induce SE, reported a significant increase in RNA oxidation in vulnerable neurons in rat brains immediately after SE followed by neuronal death. However, a daily supplement of antioxidants (coenzyme Q10) significantly reduced RNA oxidation and protected rats from SE and neuronal loss (Kurosinski and Götz, 2002).

2.4 Carbamazepine

2.4.1 Description of Carbamazepine

Carbamazepine, USP is a white to off-white powder, practically insoluble in water but soluble in alcohol and in acetone. It is an anticonvulsant and specific analgesic for trigeminal neuralgia, available for oral administration as chewable tablets usually of 100 mg and tablets usually of 200 mg. Its chemical name is 5H-dibenz[b,f]azepine-5-carboxamide, and its structural formula is $C_{15}H_{12}N_2O$ while its molecular weight is 236 (Maheswari *et al.*, 2015). In controlled clinical

trials, sold under the trade name Tegretol among others, is a medication used primarily in the treatment of epilepsy and neuropathic pain and has been shown to be effective in the treatment of psychomotor and grand mal seizures, as well as trigeminal neuralgia. It may be beneficial in glossopharyngeal neuralgia but cannot be used for the relief of trivial aches or pains since it is not a simple analgesic (Wadhawan *et al.*, 2005; Santhrani *et al.*, 2012).

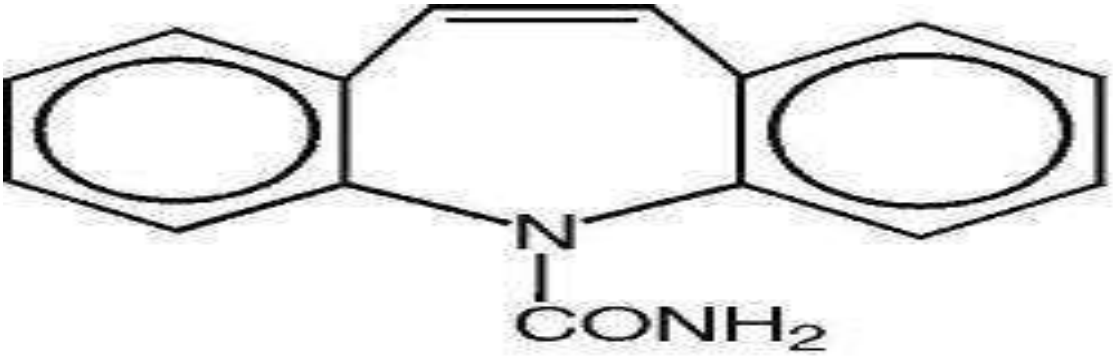

Figure 2.4: Showing the structural formula of Carbamazepine (Ambrósio *et al.*, 2002).

2.4.2 History of Carbamazepine

Carbamazepine was discovered by a chemist Walter Schindler at J.R. Geigy AG (now part of Novartis) in Basel, Switzerland, in 1953 (Scott,1993*)*. It was first marketed as a drug to treat epilepsy in Switzerland in 1963 under the brand name "Tegretol" it is used for trigeminal neuralgia (formerly known as tic douloureux) (Scott,1993*)*. It has been used as an anticonvulsant and antiepileptic in the UK since 1965, and has been approved in the US since 1974 *(Smith, 2009)*. In 1971, Drs. Takezaki and Hanaoka first used carbamazepine to control mania in patients' refractory to antipsychotics. Dr. Okuma, working independently, did the same thing with success. As they were also epileptologists, they had some familiarity with the antiaggression effects of this drug. Carbamazepine was studied for bipolar disorder throughout the 1970s *(Okuma and Kishimoto, 1998)*.

2.4.3 Mechanism of action of Carbamazepine

The mechanism of action of carbamazepine and its derivatives is relatively well understood. It has been seen to stabilize the inactivated state of voltage-gated sodium channels, making fewer of these channels available to subsequently open. This leaves the affected cells less excitable until the drug dissociates. Carbamazepine is also a GABA receptor agonist, as it has also been shown to potentiate GABA receptors made up of α_1, β_2, and γ_2 subunits. This mechanism may contribute to its efficacy in neuropathic pain and manic-depressive illness. Laboratory research has further demonstrated that it is a serotonin releasing agent and possibly even a serotonin reuptake inhibitor (*Kawata et al*., 2001).

Carbamazepine is metabolized in the liver. Cytochrome P450 3A4 was identified as the major isoform responsible for the formation of Carbamazepine-10,11-epoxide from Carbamazepine (Chi *et al.*, 2012). Human microsomal epoxide hydrolase has been identified as the enzyme responsible for the formation of the 10,11-transdiol derivative from Carbamazepine-10,11epoxide (ametabolite shown to be equipotent to Carbamazepine as an anticonvulsant in animal screens). After oral administration of 14C-Carbamazepine, 72% of the administered radioactivity was found in the urine and 28% in the feces. This urinary radioactivity was composed largely of hydroxylated and conjugated metabolites, with only 3% of unchanged carbamazepine (*Kawata et al*., 2001).

2.4.4 Contraindications and side effects of Carbamazepine

Carbamazepine should not be used in patients with a history of previous bone marrow depression, hypersensitivity to the drug, or known sensitivity to any of the tricyclic compounds,

such as amitriptyline, desipramine, imipramine, protriptyline, nortriptyline, among others (Rahangadale *et al.,* 2012). Likewise, on theoretical grounds its use with monoamine oxidase (MAO) inhibitors is not recommended. Before administration of Carbamazepine, MAO inhibitors should be discontinued for a minimum of 14 days, or longer if the clinical situation permits. This is because:

- It may cause serious and sometimes fatal dermatologic reactions like toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome (SJS).
- It may cause aplastic anemia and agranulocytosis in patients with a history of adverse hematologic reaction to any drug (Rahangadale *et al.*, 2012).
- It may cause drug reaction with eosinophilia and systemic symptoms (DRESS) or Multiorgan Hypersensitivity that could be been fatal or life-threatening (Aliyu *et al.*, 2013).
- It may cause activation of a latent psychosis and in elderly patients of confusion or agitation due to its relationship with other tricyclic compounds (Maheswari *et al.*, 2014).
- It may cause increase porphyrin precursors and may cause induction of acute attacks of hepatic porphyria (Maheswari *et al.*, 2014).
- It may also cause hepatic disturbances, ranging from slight elevations in liver enzymes to rare cases of hepatic failure that may progress despite discontinuation of the drug (Aliyu *et al.*, 2013).
- It may cause obvious oxidative stress inhibiting all antioxidant enzyme activities and reducing glutathione (GSH) content (Suleiman *et al.*, 2015).

2.5 Oxidative Stress

2.5.1 Definition of oxidative stress

The term “oxidative stress” has been used to define a state in which ROS and RNS reach excessive levels, either by excess production or insufficient removal. Thus, it is an “imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage” (Basir *et al.*, 2005)**.** At the beginning of life, the organisms obtained their energy (ATP) by anoxygenic photosynthesis, for which oxygen was toxic. Most of the metabolic pathways were developed during this anaerobic stage of life (Kelkar *et al.*, 2008). Cyanobacteria started producing oxygen from photosynthesis, which raised the atmospheric oxygen, and favored those organisms which have evolved into eukaryotic cells with mitochondria, able to use oxygen for a more efficient energy production (Basir *et al.*, 2005). Whenever a cell’s internal environment is perturbed by infections, diseases, toxins or nutritional imbalance, mitochondria diverts electron flow away from itself, forming reactive oxygen species (ROS) and reactive nitrogen species (RNS), thus lowering oxygen consumption (Kelkar *et al.*, 2008). This “oxidative shielding” acts as a defense mechanism for either decreasing cellular uptake of toxic pathogens or chemicals from the environment, or to kill the cell by apoptosis and thus avoid the spreading to neighboring cells. Therefore, ROS formation is a physiological response to stress (Basir *et al.*, 2005). Being highly reactive molecules, the pathological consequence of ROS and RNS excess is damage to proteins, lipids and DNA. Consistent with the primary role of free radicals formation, this oxidative stress damage may lead to physiological dysfunction, cell death, pathologies such as diabetes and cancer, and aging of the organism (Ahmad *et al.*, 2015).

2.5.2 Reactive oxygen species and reactive nitrogen species production

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are highly reactive molecules, which can be free radicals such as superoxide (O_2^-), hydroxyl (OH^-), peroxyl (RO_2), hydroperoxyl (HRO_2^-), nitric oxide (NO) and nitrogen dioxide (NO_2^-), or non-radicals such as hydrogen peroxide (H_2O_2), hydrochlorous acid (HOCl), peroxynitrite ($ONOO^-$), nitrous oxide (HNO_2), and alkyl peroxynitrates (RONOO). The most important ones involved in pathophysiology of diabetes and its complications are superoxide (O_2^-), nitric oxide (NO), and peroxynitrite ($ONOO^-$). There are basically two pathways for O_2^- production: NADPH oxidases and mitochondrial function, while NO and $ONOO^-$ are produced by the Nitric Oxide Synthase pathway (Birben *et al.*, 2012).

2.5.3 Biomarkers of oxidative stress

2.5.3.1 Lipid peroxidation

Hydroperoxides -that can be produced by peroxyl radicals which remove hydrogen from lipids-, can further propagate the free-radical pathway and have toxic effects on cells both directly and through degradation to highly toxic hydroxyl radicals. They may also react with transition metals like iron or copper to form stable aldehydes such as malondialdehydes that will damage cell membranes (Bisht and Sisodia, 2010).

2.5.3.2 Glutathione levels

Reduced glutathione is a major intracellular redox buffer that may approach concentrations up to 10 mM, and functions as a direct free-radical scavenger and a cosubstrate for glutathione peroxidase activity, and as a cofactor for many enzymes forming conjugates in endo- and

xenobiotic reactions (Kunwar and Priyadarsini, 2011). Its concentration is found to be decreased in the liver, kidney, pancreas, plasma, red blood cells, nerve, and precataractous lens of chemically induced diabetic animals. However, there is also some contradictory evidence of increased glutathione concentration in diabetic rat kidney and lens. It includes Glutathione Peroxidase, Glutathione Transferase and Glutathione Reductase, and enzymes that are found in the cytoplasm, mitochondria, and nucleus. Glutathione peroxidase metabolizes hydrogen peroxide to water by using reduced glutathione as a hydrogen donor. Glutathione disulfide is recycled back to glutathione by glutathione reductase, using the cofactor NADPH generated by glucose 6- phosphate dehydrogenase (Bisht and Sisodia, 2010).

2.5.3.3 Catalase activity

Catalase, located in peroxisomes, decomposes hydrogen peroxide to water and oxygen. Catalase activity is consistently found to be elevated in heart and aorta, as well as brain of diabetic rats but low in renal, hepatic and red blood cell (Kunwar and Priyadarsini, 2011).

2.5.3.4 Superoxide dismutase (SOD) activity

Isoforms of SOD are variously located within the cell. CuZn-SOD is found in both the cytoplasm and the nucleus. Mn-SOD is confined to the mitochondria, but can be released into extracellular space. SOD converts superoxide anion radicals produced in the body to hydrogen peroxide, thereby reducing the likelihood of superoxide anion interacting with nitric oxide to form reactive peroxynitrite (Jaya *et al.*, 2015).

2.5.3.5 Vitamins

Vitamins A, C, and E are diet-derived and detoxify free radicals directly. They also act in recycling processes to generate reduced forms of the vitamins. Tocopherol is reconstituted when ascorbic acid recycles the tocopherol radical; dihydroascorbic acid, which is generated, is recycled by glutathione. These vitamins also foster toxicity by producing pro-oxidants under some conditions. Vitamin E, a component of the total peroxyl radical-trapping antioxidant system, reacts directly with peroxyl, superoxide radicals and singlet oxygen, and protects membranes from lipid peroxidation. The deficiency of vitamin E is concurrent with increased peroxides and aldehydes in many tissues (Hideaki *et al.*, 1999).

2.5.3.6 Nitric oxide level

Generally, nitric oxide at physiological levels produces many benefits to the vascular system. But increased oxidative stress and subsequent activation of the transcription factor NF-kappa B have been linked to the development of late diabetic complications, by enhancing nitric oxide production, which is believed to be a mediator of islet β-cell damage. Nitric oxide may react with superoxide anion radical to form reactive peroxyl nitrite radicals. On the other hand, nitric oxide levels in plasma are decreased in alloxan-diabetic rats, an effect that can be abrogated by prior and simultaneous administration of L-arginine, a precursor of nitric oxide (Balcer *et al.*, 2012).

2.5.4 Effects of oxidative stress in human health

Human body works against oxidative stress induced by free radicals through a variety of defense mechanisms such as anticipatory mechanism, tissue repair, physical resistance and antioxidant defenses (Bisht and Sisodia, 2010). ROS derived reactions of various free radicals are involved

in the development of numerous human illnesses including Neurodegenerative disorders (e.g. Alzheimer's disease and Parkinsonism), Cardiovascular diseases (e.g. atherosclerosis and high blood pressure), Respiratory diseases (e.g. bronchial asthma), Renal disorders (e.g. glomerulonephritis and uremia), autoimmune disease (e.g. rheumatoid arthritis), peptic ulcers, lung cancers, cataract and retinopathy (Patel and Sharma, 2014).

2.5.5 Antioxidant defense in organism

As a small part the oxygen consumed for aerobic processes will be converted into superoxide anion, which will have to be scavenged or converted into less reactive (and less harmful) molecules. The main enzymes that regulate this process are Superoxide dismutase (SOD), Glutathione Peroxidase (GSH-Px) and Catalase. SOD is considered a first-line defense against ROS. GSH-Px catalyzes degradation of H_2O_2 by reduction, where two gluthathione (GSH) molecules are oxidized to glutathione disulfide (GSSG). Regeneration of GSH by GSH-reductase, requires NADPH, which is oxidized to $NADP^+$. Catalase, on the other hand detoxifies the H_2O_2 that diffuses from the mitochondria to the cytosol, converting it into water and molecular oxygen. (Amina *et al.*, 2015)

There are also nonenzymatic antioxidant, which mostly help regenerate GSSG back into GSH, and it include glutathione, vitamins such as A, C, E, alpha-lipoic acid, among others, that work together to eliminate oxidants from the cell. (Constantin and Octavian, 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Animals

Thirty five adult male Wistar rats were used for the study. The animals were purchased from the Animal House of the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria. They were kept in laboratory cages in the Animal House of the Department of Human Physiology having free access to standard animal feed and water *ad libitum*. The animals were divided into seven groups of five (n=5). All administrations were done orally by gavage for forty five days (Thakur, *et al*.,2012; Maheswari, *et al*., 2015).

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Group I: Rats were treated with distilled water 1ml/kg orally for forty five days.

Group II: Rats were treated with corn oil 2ml/kg orally for forty five days (Ambali *et al.,* 2010).

Group III: Rats were treated with carboxymethylcellulose 10g/L orally for forty five days (Juan, 2002).

Group IV: Rats were treated with carbamazepine 50mg/kg orally for forty five days (Thakur, *et al*., 2012).

Group V: Rats were treated with carbamazepine 50mg/kg and vitamin E 200mg/kg (Maheswari, *et al*., 2015).

Group VI: Rats were treated with carbamazepine 50mg/kg and resveratrol 20mg/kg orally for forty five days (Rai *et al.,* 2013).

Group VII: Rats were treated with carbamazepine 50mg/kg, and co-administration of vitamin E (200mg/kg) and resveratrol (20mg/kg) orally for forty five days.

3.2 Drugs/chemicals and reagents

Vitamin E (Greenbrier International, INC. USA),

Resveratrol (Mega Resveratrol Candlewood Stars Limited),

Carboxymethylcellulose, Carbamazepine (Micro Labs Limited. India),

Corn oil,

Chloroform,

Cotton wool,

Digital weighing balance,

Test tubes, plain tubes and EDTA bottles

All chemicals used were of analytical grade.

3.3 Blood Collection and Assessments of Haematological Parameters

The animals were sacrificed 24 hours after last administration. They were anaesthetized by chloroform inhalation in a closed chamber and dissected, and blood samples were collected via cardiac puncture in the laboratory of the Department of Human Physiology. Blood sample (2 ml) was collected into EDTA bottles containing 8 mg of disodium salt of ethylene diamine tetra-acetic acid. This was used for total blood count that include Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Platelet count, Packed Cell Volume (PCV) which were determined according to the method described by Dacie and Lewis (1991). 3ml of blood from the blood sample was collected into plain test tubes. This was allowed to clot and the serum was separated by centrifugation using the Denley BS400 centrifuge (England) at 3000 rpm for 10 minutes and the supernatant (serum) collected was used for the assessment of antioxidant enzyme activity and lipid peroxidation.

3.4 Assessment of Antioxidant Enzymes and Lipid Peroxidation

Catalase (CAT)

Catalase (CAT) activity was measured using Abebi's method (1974). Exactly 10µl of serum was added to a test tube containing 2.80ml of 50mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1ml of freshly prepared 30mM H_2O_2 and the decomposition rate of H_2O_2 was measured at 240nm for 5 minute on a spectrophotometer. A molar extinction coefficient (E) of 0.041mM^{-}1 -cm^{-}1 was used to calculate the Catalase activity. Catalase Conc.= Absorbance/E. Catalase Activity = Catalase Con./Protein Conc. (mg/ml)

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) was determined by the method described by Fridovich (1989).

Principle: The ability of superoxide dismutase (SOD) to inhibit antioxidation of adrenaline at pH 10.2 form the basis of this assay.

Reagents: 0.05M carbonate buffer: 114.3g of Na_2CO_3 and 4.2g of $NaHCO_3$ as dissolved in distilled water and make up to 1000ml in a volumetric flask. The buffer was adjusted to pH 10.2.0.3mM Adrenaline: 0.01g of adrenaline as dissolved in 17ml of distilled water, the solution was prepared fresh.

Procedure: Tissue homogenate of 0.1ml was diluted in 0.9ml of distilled water to make 1:10 dilution of micro some. An aliquant mixture of 0.2ml of the diluted micro some was added to 2.5ml of 0.05M carbonate buffer. The reaction was started with the addition of 0.3ml of 0.3mM Adrenaline. The reference mixture contained 2.5ml of 0.05M carbonate buffer, 0.3ml of 0.3mM Adrenaline and 0.2ml of d distilled water. The Absorbance was measured over 30 seconds up to 150 seconds at 480nm.

Calculations: Increase in absorbance per minute = $(A_2 - A_1)/2.5$

% Inhibition = 100 - {(Incr. in absorbance for sample/Incr. in absorbance of blank) x 100}

1 unit of SOD activity is the quantity of SOD necessary to elicit 50% inhibition of the oxidation of Adrenaline to adenochrome in 1 minute.

Glutathion Peroxidase (GPx)

Glutathion peroxidase activity was determined by the method of Ellman (1959). 1 ml of supernatant (0.5 ml plasma precipitated by 2 ml of 5% TCA) was taken and 0.5 ml of Ellman's reagent (0.0198% DTNB in 1% sodium citrate) and 3 ml of phosphate buffer (pH 8.0) was added. The colour developed was read at 412 nm in plasma was estimated colorimetrically by measuring malondialdehyde (MDA) by the method of Albro *et al.,* (1986) and Das *et al.,* (1990). In brief, 0.1 ml of plasma was treated with 2 ml of (1:1:1 ratio) TBA–TCA–HCL reagent (TBA 0.37%, 0.25N HCL and 15% TCA) and placed in water bath for 15 min, cooled and centrifuged and then clear supernatant was measured at 535nm against reference blank.

Malondialdehyde (MDA)

Lipid peroxidation in plasma was estimated colorimetrically by measuring malondialdehyde (MDA) by the method of Albro *et al*. (1986) and Das *et al*. (1990). In brief, 0.1 ml of plasma was treated with 2 ml of (1:1:1 ratio) TBA–TCA–HCL reagent (TBA 0.37%, 0.25N HCL and 15% TCA) and placed in water bath for 15 min, cooled and centrifuged and then clear supernatant was measured at 535nm against reference blank.

3.5 Statistical Analysis

All data generated were expressed as mean ± standard of error mean (Mean ± S.E.M.). The data obtained were analyzed using one-way analysis of variance (ANOVA), followed by *Tukey's* post-hoc test to compare the level of significance between groups using SPSS version 20. Values of $P<0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of Vitamin E and Resveratrol on Oxidative Stress Biomarkers

4.1.1 Effect of Vitamin E and Resveratrol on Lipid Peroxidation

Figure 4.1 shows the results on the effect of vitamin E and resveratrol on malondialdehyde (MDA) concentration in CBZ-induced oxidative stress. The result showed that treatment with Carbamazepine significantly ($P<0.01$) increased MDA serum level to 1.42 ± 0.04µmol/L when compared to that of normal control 0.92 ± 0.04µmol/L. However, treatment with vitamin E and resveratrol was seen to significantly ($P < 0.01$) reduce CBZ-induced increase in MDA serum level to 1.02 ± 0.05µmol/L and 1.00 ± 0.03 µmol/L respectively, in comparison to CBZ-treated group. The result also showed that co-administration of vitamin E and resveratrol significantly ($P<0.01$) reduced CBZ-induced increase in MDA serum level (0.98 ± 0.05µmol/L) in comparison to that of CBZ-treated group.

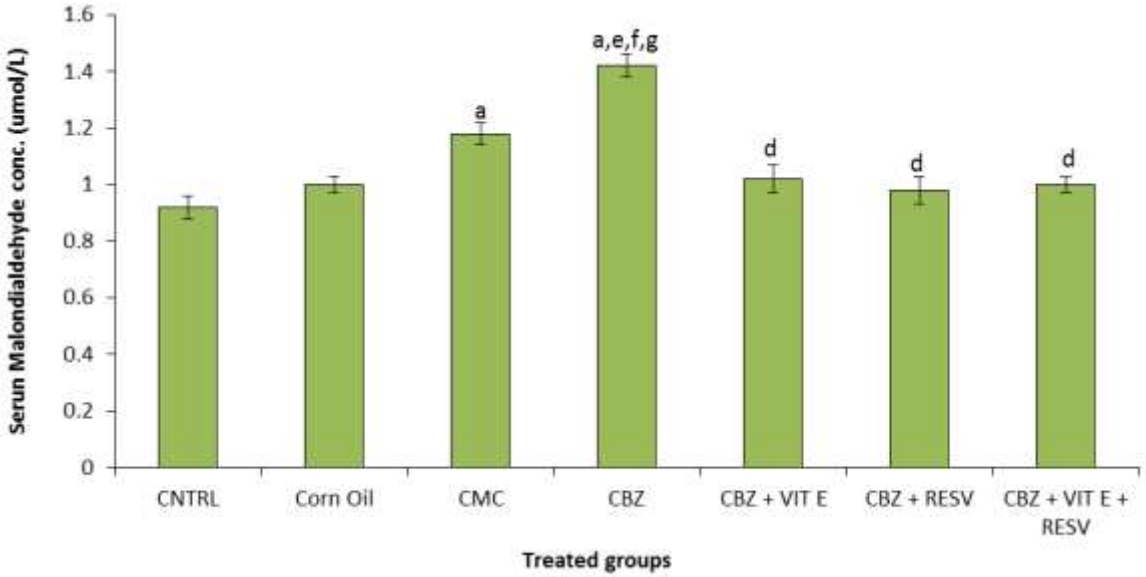

Figure 4.1: Effect of Vitamin E and Resveratrol on serum level of Malondialdehyde. CNTRL= Control; CMC= Carboxymethylcellulose; CBZ= Carbamazepine; VIT E= Vitamin E; RESV= Resveratrol. Superscripts **a,d,e,f**, and **g** indicate statistical significant difference ($P<0.01$) when compared to CNTRL, CBZ, CBZ+VIT E, CBZ+RESV and CBZ+VIT E + RESV treated groups respectively.

4.1.2 Effect of Vitamin E and Resveratrol on Superoxide Dismutase activity

Figure 4.2 shows the results on the effect of vitamin E and resveratrol on superoxide dismutase (SOD) activity in CBZ-induced oxidative stress. The result showed that treatment with Carbamazepine significantly ($P<0.01$) decreased SOD activity to 2.04 ± 0.02 IU/L in comparison to that of the normal control (2.40 ± 0.03IU/L). However, treatment with vitamin E and resveratrol significantly ($p< 0.01$) increased SOD activity to 2.42 ± 0.05IU/L and 2.48 ± 0.05IU/L respectively, in comparison to CBZ-treated group. Furthermore, co-administration of vitamin E and resveratrol significantly ($p<0.01$) increased SOD activity to 2.52 ± 0.04IU/L in comparison to that of CBZ-treated group

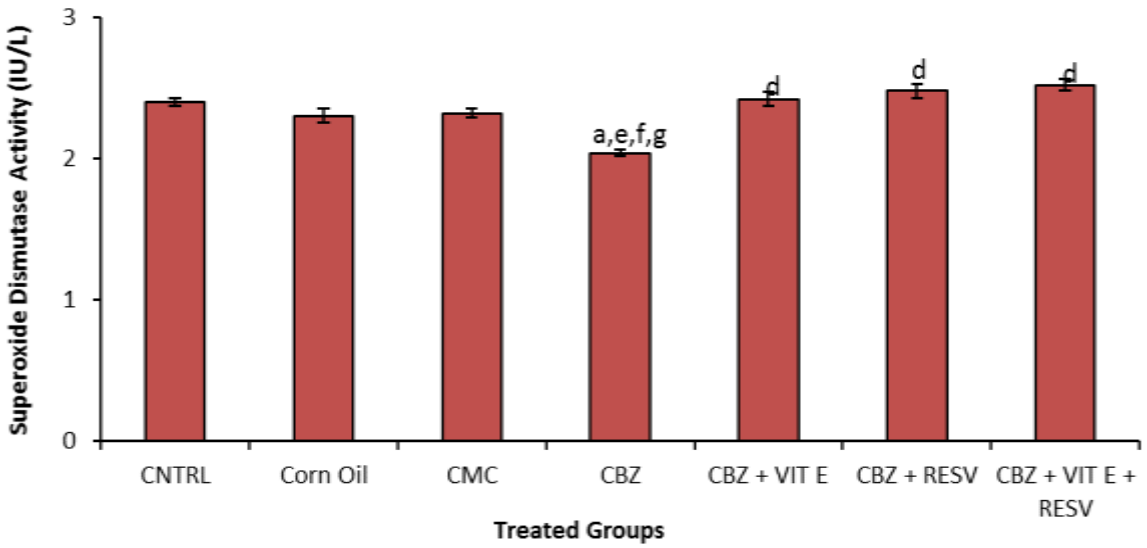

Figure 4.2: Effect of Vitamin E and Resveratrol on serum level of Superoxide Dismutase CNTRL= Control; CMC= Carboxymethylcellulose; CBZ= Carbamazepine; VIT E= Vitamin E; RESV.= Resveratrol. Superscripts **a,d,e,f** and **g** indicate statistical significance (P< 0.01) when compared to CNTRL, CBZ, CBZ+VIT E, CBZ+RESV and CBZ+VIT E + RESV treated groups respectively.

4.1.3 Effect of Vitamin E and Resveratrol on Catalase activity

Figure 4.3 shows the results on the effect of vitamin E and resveratrol on catalase (CAT) activity in CBZ-induced oxidative stress. The result revealed that treatment with Carbamazepine significantly ($P<0.01$) decreased CAT activity (46.60 ± 0.40IU/L) in comparison to that of the normal control (52.00 ± 0.32IU/L). However, treatment with vitamin E and resveratrol significantly ($p< 0.01$) increased CAT activity to (51.80 ± 0.49IU/L) and (53.20 ± 0.37 IU/L) respectively, in comparison to CBZ-treated group. Furthermore, co-administration of vitamin E and resveratrol significantly ($p<0.01$) increased CAT activity to 54.00 ± 0.31IU/L in comparison to that of CBZ-treated group

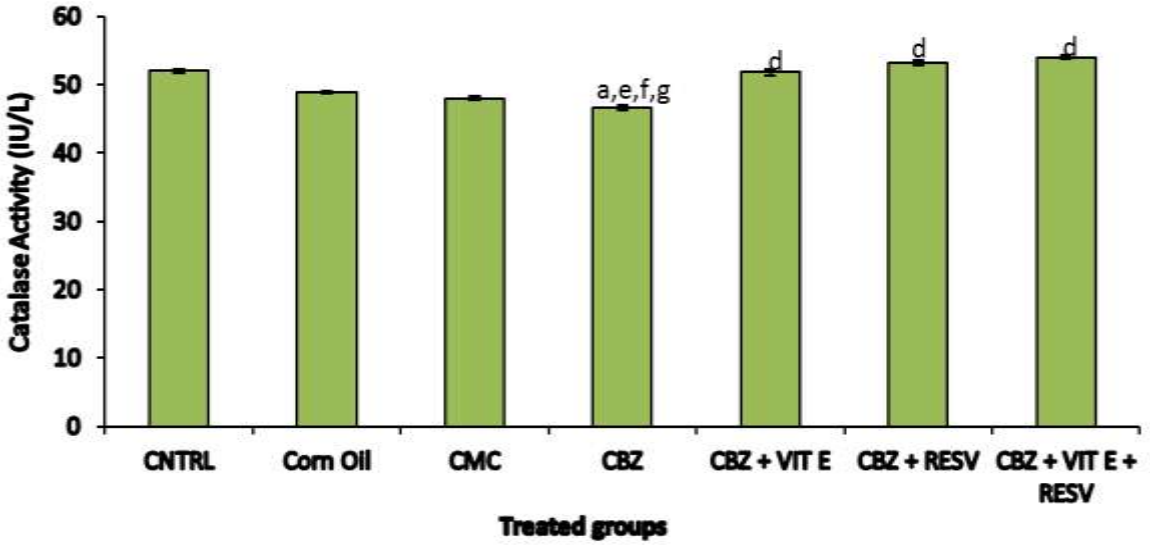

Figure 4.3: Effect of Vitamin E and Resveratrol on serum level of Catalase. CNTRL= Control; CMC= Carboxymethylcellulose; CBZ= Carbamazepine; VIT E= Vitamin E; RESV.= Resveratrol. Superscripts **a, d, e, f**, and **g** indicate statistical significant difference P (< 0.01) when compared to CNTRL, CBZ, CBZ+VIT E, CBZ+RESV, CBZ+VIT E+RESV treated groups respectively.

4.1.4 Effect of Vitamin E and Resveratrol on Glutathione Peroxidase serum level

Figure 4.4 shows the results on the effect of vitamin E and resveratrol on Glutathione Peroxidase GPx serum level in CBZ-induced oxidative stress. The result showed that treatment with Carbamazepine significantly ($P<0.01$) decreased GPx serum level to 42.20 ± 0.49IU/L in comparison to that of the normal control 45.20 ± 0.37IU/L. Treatment with vitamin E and resveratrol significantly ($p< 0.01$) increased GPx serum level to 47.60 ± 0.40 IU/L and 48.80 ± 0.37 IU/L respectively, in comparison to CBZ-treated group. Furthermore, co-administration of vitamin E and resveratrol significantly ($p<0.01$) increased GPx serum level to 49.20 ± 0.66 IU/L in comparison to that of CBZ-treated group

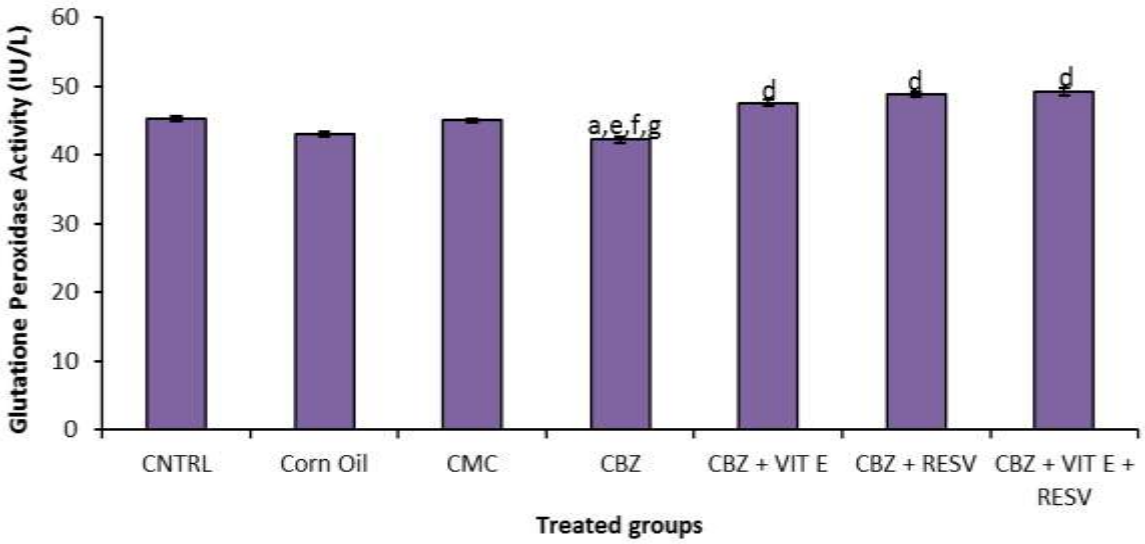

Figure 4.4: Effect of Vitamin E and Resveratrol on serum level of Glutathione Peroxidase. CNTRL= Control; CMC= Carboxymethylcellulose; CBZ= Carbamazepine; VIT E= Vitamin E; RESV.= Resveratrol. Superscripts **a,d,e,f** and **g** indicate statistical significance (P< 0.01) when compared to CNTRL, CBZ, CBZ+VIT E, CBZ+RESV, CBZ+VIT E+RESV treated groups respectively.

4.2 Effect of Vitamin E and Resveratrol on Haematological parameters

4.2.1 Effect of Vitamin E and Resveratrol on Red Blood Cell Count.

Figure 4.5 shows the result on the effect of vitamin E and Resveratrol on RBC counts in carbamazepine induced oxidative stress in male wistar rats. The result showed treatment with CBZ significantly $P< 0.05$ decreased RBC counts to $6.85\pm 0.28x10^{12}$/L in comparison to that of the normal control ($8.00\pm0.19x10^{12}$/L). Following treatment with vitamin E, the result showed non-significant decrease in RBC count ($6.84\pm0.26x10^{12}$/L) when compared to CBZ treated group, however, treatment with Resveratrol significantly $P<0.05$ increased RBC count ($8.14\pm0.21.x10^{12}$/L) in comparison to CBZ treated group. Co-administration of vitamin E and Resveratrol showed significant ($P<0.05$) increase in RBC count ($8.53\pm0.15x10^{12}$/L) in comparison to that of CBZ – treated group.

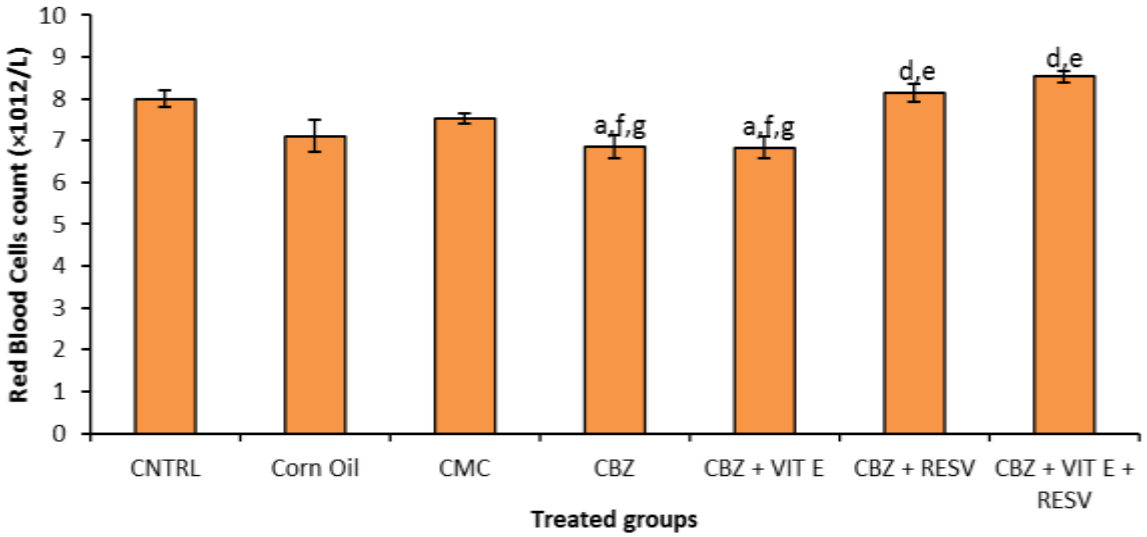

Figure 4.5: Effect of Vitamin E and Resveratrol on Red Blood Cell Count. CNTRL= Control; CMC= Carboxymethylcellulose; CBZ= Carbamazepine; VIT E= Vitamin E; RESV= Resveratrol. Superscripts **a,d,e,f** and **g** indicate statistical significance (P< 0.05) when compared to CNTRL, CBZ, CBZ+VIT E, CBZ+RESV, CBZ+VIT E+RESV treated groups respectively.

4.2.2 Effect of Vitamin E and Resveratrol on Packed Cell Volume.

Figure 4.6 shows the results on the effect of vitamin E and resveratrol on Packed Cell Volume (PVC) in CBZ-induced oxidative stress. The result shows treatment with CBZ revealed highly significant $P<0.01$ decrease in PCV ($39.08\pm1.66\%$) when compared to that of the normal control (50.04 ± 1.14). Treatment with vitamin E, showed increased PCV ($42.40\pm0.76\%$) but not statistically significant when compared to CBZ treated group, however, treatment with resveratrol and co-administration of vitamin E and resveratrol revealed highly significant $P<0.01$ increase in PCV level to $48.88\pm1.43\%$ and $50.70\pm0.86\%$ in comparison to CBZ treated group respectively.

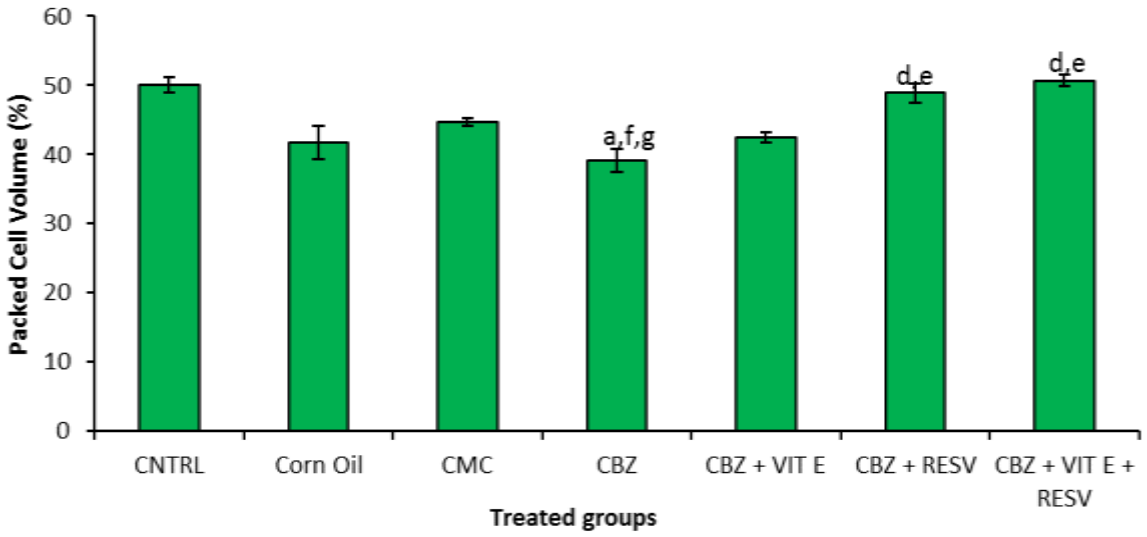

Figure 4.6: Effect of Vitamin E and Resveratrol on Packed Cell Volume. CNTRL= Control; CMC= Carboxymethylcellulose; CBZ= Carbamazepine; VIT E= Vitamin E; RESV= Resveratrol. Superscripts **a,d,e,f** and **g** indicate statistical significance ($P < 0.01$) when compared to CNTRL, CBZ, CBZ+VIT E, CBZ+RESV, CBZ+VIT E+RESV treated groups respectively.

4.2.3 Effect if Vitamin E and Resveratrol on Platelets Count.

Figure 4.7 shows the results on the effect of vitamin E and resveratrol on platelets count in CBZ – induced oxidative stress. The result revealed that treatment with carbamazepine significantly ($P<0.05$) decreased platelets count ($256.00+12.20x10x^{9}/L$) in comparison to that of the normal control ($314.00\pm7.07x10^{9}/L$). Following treatment with vitamin E and resveratrol, the result revealed significant ($P<0.05$) increase in platelet count to $323.20 \pm 23.83x10^{9}/L$ and $338.20 \pm 3.98\ x10^{9}/L$ respectively when compared to CBZ treated group. Co-administration of vitamin E and resveratrol revealed highly significant ($P<0.01$) increase in platelet count ($361.80\pm80.46x10^{9}/L$) in comparison to that of CBZ treated group.

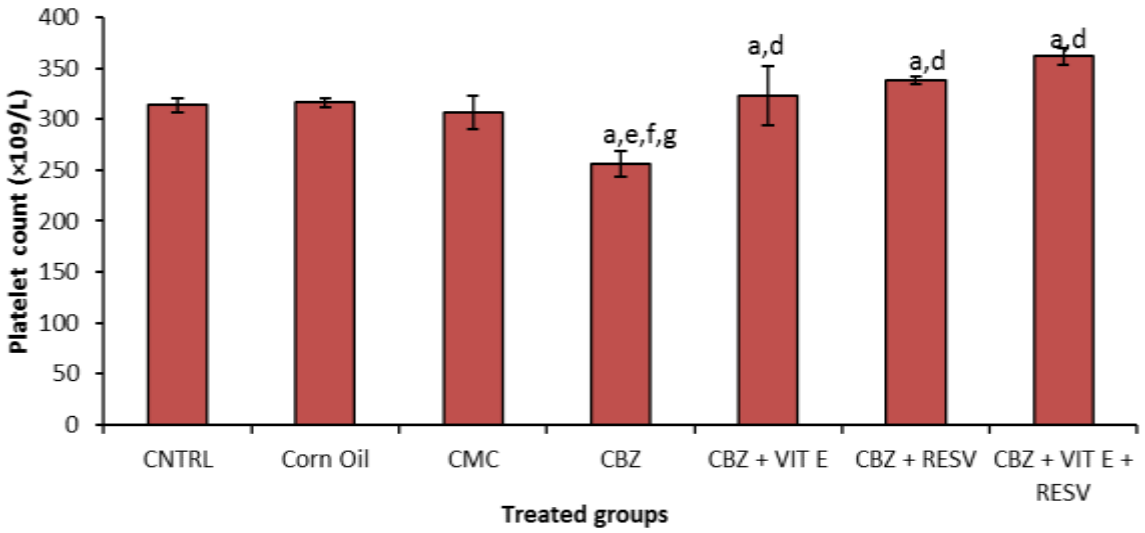

Figure 4.7: Effect of Vitamin E and Resveratrol on Platelet count. CNTRL= Control; CMC= Carboxymethylcellulose; CBZ= Carbamazepine; VIT E= Vitamin E; RESV= Resveratrol. Superscripts **a,d,e,f** and **g** indicate statistical significance (P< 0.01) when compared to CNTRL, CBZ, CBZ+VIT E, CBZ+RESV, CBZ+VIT E+RESV treated groups respectively.

4.2.4 Effect of Vitamin E and Resveratrol on White Blood Cells Count.

Figure 4.8 shows the results on the effect of vitamin E and resveratrol on White Blood Cells (WBC) count in CBZ – induced oxidative stress. The result shows that treatment with carbamazepine significantly $P<0.01$ decreased WBCs count ($4.20\pm0.21x10^9$/L) in comparison to that of the normal control group ($6.08\pm0.31x10^9$/L). Following treatment with vitamin E and resveratrol, the result showed an increase in WBC counts $4.80 \pm 0.41x10^9$/L and $5.32 \pm 0.20x10^9$/L respectively when compared to CBZ-treated group. Furthermore, co-administration of vitamin E and resveratrol showed a high significant increase in WBCs count (5.90 ± 0.18 $x10^9$/L) in comparison to CBZ – treated group.

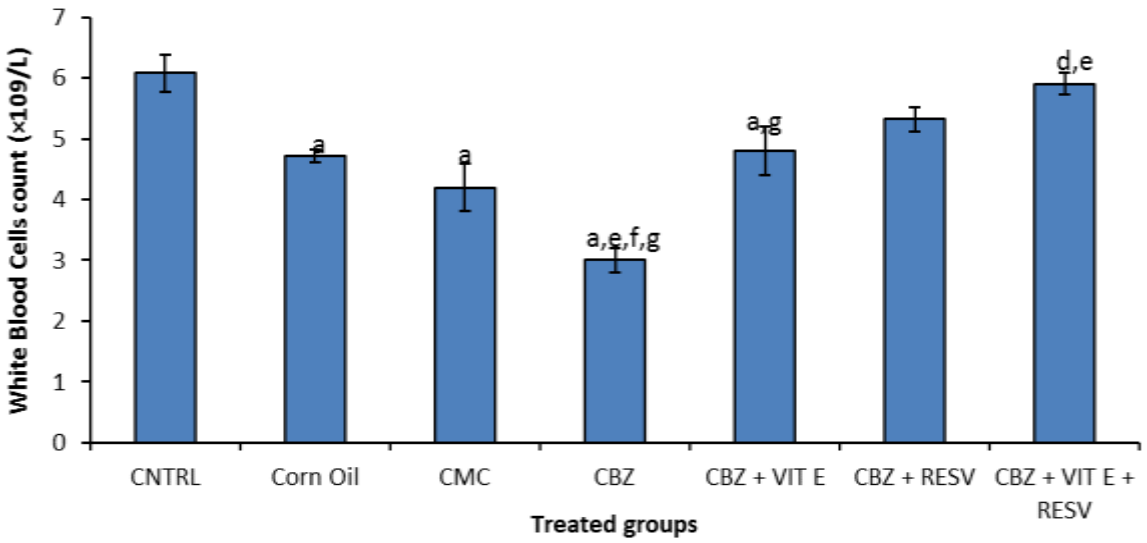

Figure 4.8: Effect of Vitamin E and Resveratrol on White Blood Cell count. CNTRL= Control; CMC= Carboxymethylcellulose; CBZ= Carbamazepine; VIT E= Vitamin E; RESV= Resveratrol. Superscripts **a,d,e,f** and **g** indicate statistical significance (P< 0.01) when compared to CNTRL, CBZ, CBZ+VIT E, CBZ+RESV, CBZ+VIT E+RESV treated groups respectively.

CHAPTER FIVE

5.0 DISCUSSION

The present study investigated the effect of vitamin E and resveratrol on carbamazepine induced oxidative stress in male Wistar rats.

The study revealed that treatment with Carbamazepine significantly (P <0.01) increased MDA serum level when compared to that of normal control. The rise MDA serum level may be due to intermediate free radicals formed during carbamazepine administration. The reactive oxygen species in the absence of an efficient defense mechanism cause peroxidation of membrane poly-unsaturated fatty acids. Lipid peroxidation occurs in areas where polyunsaturated fatty acid side chains are prevalent. These chains react with O_2 creating the peroxyl radical, which can obtain H^+ from another fatty acid, creating a continuous reaction which result in increased plasma lipid peroxidation. This finding is in agreement with the reports of Imad, (2012), Thakur *et al.,* (2012), and Maheswari *et al.,* (2015), who revealed that carbamazepine causes oxidative stress as represented by elevated serum malondialdehyde (MDA) level. However, treatment with vitamin E and resveratrol was seen to significantly ($p < 0.01$) reduce CBZ-induced increase in MDA serum level in comparison to CBZ-treated group. This could be due to the antioxidant effect of vitamin E and resveratrol to prevent lipid peroxidation in cellular membrane (Azzi *et al*., 1992) thereby reducing MDA serum level. This result is in agreement with Maheswari *et al*., (2015) who reported treatment with vitamin E showed reduction in the levels of carbamazepine induced lipid peroxidation. Co-administration of vitamin E and resveratrol also significantly ($p <0.01$) reduced MDA serum level.

In the present study, treatment with Carbamazepine significantly (P<0.01) decreased SOD activity, CAT activity and GPx serum level in comparison to that of the normal control. This could have been due to inhibition of antioxidant enzyme actions by carbamazepine which result in oxidative stress or the consistent depletion of the endogeneous antioxidant enzymes which are being used to ameliorate or combat the existing oxidative stress resulting in subsequent decrease in oxidative stress bio-makers. This is in agreement with the findings of Thakur, *et al*., (2012) and Maheswari *et al*., (2015) who reported that carbamazepine treatment decreased the levels of superoxide dismutase, catalase, and glutathione peroxidase. Suleiman *et al*., (2015) also reported that CBZ induced oxidative stress in the testis of wistar rats.

Following treatment with vitamin E and resveratrol SOD activity, CAT activity and GPx serum level was seen to be significantly ($p< 0.01$) increased in comparison to CBZ-treated group. This could be due to the ability of vitamin E to prevent chain initiation and propagation of free radicals thereby decreasing reactive oxygen species which result in increasing the level of SOD activity, CAT activity and GPx serum level (Azzi *et al*., 1992). Also, it could be due to the lipophilic character of resveratrol which enable it to bind the lipoprotein particles thereby improving its antioxidant activity (Belguendouz *et al*., 1998). The antioxidant effect of resveratrol could also be attributed to the blockade of radical propagation by formation of a resonance-stabilized peroxy radical (Kovacic and Somanathan, 2010).

Co-administration of vitamin E and resveratrol significantly ($p<0.01$) increased SOD activity CAT activity and GPx serum level in comparison to that of CBZ-treated group This could be due to augmentative effect of the combine treatment thereby converting the pro-oxidative property of carbamazepine by up-regulating the activities of the endogenous anti-oxidant enzymes. This is in

agreement with the findings of Goldfarb *et al*.,(2005) who found out that combined antioxidants attenuate the rise or increase in blood biomarkers of oxidative stress in non-resistance trained females. It is also in agreement with the findings of Chanvitayapongs *et al*., (1997) who found out that the combination of resveratrol and vitamin C and or vitamin E was more effective in protecting the cell from oxidative stress induced by addition of Fe^{2+} and t butyl hydroperoxide to cultured PC12 cell medium than any of the three oxidants alone. Suleiman *et al.,* (2015) also reported that *Hibiscus sabdariffa* and vitamin E or their combination ameliorated CBZ induced oxidative stress in the testis of wistar rats.

From the present study, the result on total blood count revealed treatment with CBZ significantly decreased RBC counts, PCV, PLT and WBC count in comparison to that of the normal control. This may be due to the fact that carbamazepine undergoes oxidative metabolism which result in the formation of a toxic arene oxide intermediate. The oxide covalently binds with cell macromolecules causing bone marrow toxicity and aplastic anaemia (Thakur *et al*., 2011). It could also be due to the oxidative stress developed from the administration of CBZ resulting in lipid peroxidation of the lipid bi-layer of cells and subsequent compromise of cellular membrane integrity. This is in agreement with the findings of Thakur, *et al*., (2012) who reported that carbamazepine produced a marked decrease in hemoglobin, total erythrocyte count, total leucocyte count, differential leucocyte count, platelet count and packed cell volume.

Following treatment with vitamin E, the result showed non-significant decrease in RBC count, but there was increase in PCV and WBC count though not statistically significant when compared to CBZ treated group. However, there was significant increase in PLT in comparison to CBZ treated group. The non-significant decrease in RBC count may be associated with low

dose of Vitamin E. The increase in PCV, PLT and WBC may be due ability of Vitamin E to scavenge lipid peroxyl radicals leading to decrease in lipid peroxidation. Treatment with resveratrol significantly increased RBC, PCV, PLT and non-significant increase in WBC counts, in comparison to CBZ treated group. This could be due to ability of Resveratrol to activate PARP 1 and this stimulate network of genes that protect cells from oxidative-induced damage (Sajish and Schimmel , 2015). This result is in agreement with Nurgül *et* al., (2014) who investigated the protective effects of resveratrol on hematological and biochemical changes in rats induced by sodium fluoride and they found out that hematological changes were ameliorated in the group that received fluoride and resveratrol. However is not in agreement with the findings of Juan *et al*., (2002), who reported that high-dose of resveratrol administration did not alter hematological parameters. Furthermore, co-adminstration of Vitamin E and Resveratrol significantly increased RBC, PCV, PCV, and WBC count in comparison to that of CBZ treated group. This could have been due to the antioxidant activities of Vitamin E and Resveratrol to obliterate lipid peroxidation resulting in reduced oxidative stress as well as the destruction of the blood cells. This result is in agreement with Alhassan *et al*., (2010) who reported that co-administration of antioxidants showed an improvement in packed cell volume, hemoglobin concentration and red blood cell in rats subjected to heat stress.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The result of the present study signifies that carbamazepine decreased the levels of oxidative stress bio-makers (SOD, CAT and GPx), full blood count (RBCs, PCV, PLT, and WBCs) whereas it increased MDA levels. Administration of vitamin E (200 mg/kg) and resveratrol (20 mg/kg) or their combination improved oxidative stress bio-makers (SOD, CAT and GPx), full blood count (RBCs, PCV, PLT, and WBCs) and reduced MDA level. The results support that vitamin E (200 mg/kg) and resveratrol (20 mg/kg) ameliorated carbamazepine induced oxidative stress in rats. The effects of these antioxidants are considered to be related to their intrinsic ability to scavenge free radicals.

6.2 Recommendations

1. The co-administration of Vitamin E and Resveratrol could be adopted to help alleviate carbamazepine induced oxidative stress.
2. Further research is recommended to compare higher doses of vitamin E and resveratrol.
3. Further research on tissue morphology is also suggested coupled with lipid profiles

6.4 Contributions to Knowledge

1. Co-administration of vitamin E (200mg/kg) and resveratrol (20mg/kg) reduces CBZ-induced increase in MDA serum level (0.98 ± 0.05 µmol/L vs 1.42± 0.04) while increasing the level of endogenous anti-oxidant enzymes; S0D (2.52 ± 0.04 vs 2.04 ± 0.02), CAT (54.00 ± 0.66 vs 46.60 ± 0.40) and GPx (49.20 ± 0.66 vs 42.20 ± 0.49).

2. Co-administration of vitamin E (200 mg/kg) and resveratrol (20 mg/kg) increases RBC ($8.53 \pm 0.15 \times 10^{12}$/L vs $6.85 \pm 0.28 \times 10^{12}$/L), PCV (50.70 ± 0.86 vs 39.08 ± 1.66), PLT ($361.80 \pm 8.46 \times 10^{9}$/L vs $256.00 \pm 12.20 \times 10^{9}$/L) and WBC ($5.90 \pm 0.18 \times 10^{9}$/L vs $4.20 \pm 0.21 \times 10^{9}$/L).

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APPENDICES

Appendix I

Effect of vitamin E and resveratrol on oxidative stress biomarkers in CBZ-induced oxidative stress

Group	**MDA**(µmol/L)	**SOD**(IU/L)	**CAT**(IU/L)	**GPx**(IU/L)
Group 1: DW 1 ml/kg	0.92 ± 0.04	2.40 ± 0.03	52.00 ± 0.32	45.20 ± 0.37
Group 2: CO 2 ml/kg	1.00 ± 0.03	2.30 ± 0.03	48.80 ± 0.20	43.00 ± 0.32
Group 3: CMC 10 g/L	1.18 ± 0.04	2.32 ± 0.04	48.00 ± 0.32	45.00 ± 0.32
Group 4: CBZ 50 mg/kg	1.42 ± 0.04*	2.04 ± 0.02*	46.60 ± 0.40*	42.20 ± 0.49*
Group 5:CBZ 50 mg/kg + Vit. E 200 mg/kg	1.02 ± 0.05*	2.42 ± 0.05*	51.80 ± 0.49*	47.60 ± 0.40*
Group 6: CBZ 50 mg/kg + Resv. 20 mg/kg	1.00 ± 0.03*	2.48 ± 0.05*	53.20 ± 0.37*	48.80 ± 0.37*
Group 7: CBZ 50 mg/kg + Vit. E 200 mg/kg + Resv. 20 mg/kg	0.98 ± 0.05*	2.52 ± 0.04*	54.00 ± 0.31*	49.20 ± 0.66*

Significant values: $p < 0.01$ = *

DW: Distilled water; CO: Corn oil; CMC: Carboxymethylcellulose; CBZ: Carbamazepine; Vit. E: Vitamin E; Resv.: Resveratrol.

Appendix II

Effect of vitamin E and resveratrol on haematological parameters in CBZ-induced oxidative stress

Group	**RBCs** ($\times 10^{12}$/L)	**PCV** (%)	**PLT** ($\times 10^{9}$/L)	**WBCs** ($\times 10^{9}$/L)
Group 1: DW 1 ml/kg	8.00 ± 0.19	50.04 ± 1.14	314.00 ± 7.07	6.08 ± 0.31
Group 2: CO 2 ml/kg	7.11 ± 0.38	41.74 ± 2.46	316.20 ± 4.18	4.72 ± 0.10
Group 3: CMC 10 g/L	7.53 ± 0.12	44.76 ± 0.57	307.00 ± 16.32	3.00 ± 0.39
Group 4: CBZ 50 mg/kg	6.85 ± 0.28*	39.08 ± 1.66**	256.00 ± 12.20*	4.20 ± 0.21**
Group 5: CBZ 50 mg/kg + Vit. E 200 mg/kg	6.84 ± 0.26	42.40 ± 0.76	323.20 ± 23.83 *	4.80 ± 0.41
Group 6: CBZ 50 mg/kg + Resv. 20 mg/kg	8.14 ± 0.21*	48.88 ± 1.43**	338.20 ± 3.98*	5.32 ± 0.20
Group 7: CBZ 50 mg/kg + Vit. E 200 mg/kg + Resv. 20 mg/kg	8.53 ± 0.15*	50.70 ± 0.86**	361.80 ± 8.46**	5.90 ± 0.18**

Significant values: $p< 0.05$ = *; $p< 0.01$ = **

DW: Distilled water; CO: Corn oil; CMC: Carboxymethylcellulose; CBZ: Carbamazepine; Vit. E: Vitamin E; Resv.: Resveratrol.