AMELIORATIVE EFFECT OF RESVERATROL ON LEAD-INDUCED ORGAN TOXICITY IN WISTAR RATS

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NOVEMBER, 2015

DECLARATION

I declare that the work in this dissertation, entitled 'Ameliorative effect of Resveratrol on Lead induced Organ Toxicity in Wistar Rats' has been performed by me in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria under the supervision of Dr. N.M Danjuma and Dr. Musa Aliyu. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this dissertation was previously presented for another degree or diploma at any University.

SALISU Muhammad Highab

_____	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled "AMELIORATIVE EFFECT OF RESVERATROL ON LEAD-INDUCED ORGAN TOXICITY IN WISTAR RATS by Muhammad Highab SALISU, meets the regulations governing the award of the degree of Master of Science in Pharmacology, of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

DR. N.M. DANJUMA Chairman, Supervisory Committee,	_____ (Signature)	_____ Date
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DEDICATION

This dissertation is dedicated to my parents (Lt) Sidi Muhammad Salisu Muye, Hajiya Fatima Musa Muhammad and my sister Hajiya Rakiya Sidi, for their valuable support and prayers throughout my endeavours and to the entire Pharmacologists in ABU Zaria.

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ABSTRACT

Resveratrol is a potent antioxidant found abundantly in grapes and in lesser quantities in peanuts, fruits and other food items. Dozens of reports have shown that resveratrol prevents or slows the progression of a wide variety of illnesses including cancer (colon and melanoma). The aim of this experiment was to investigate the ameliorative effect of resveratrol on lead-induced organ toxicity in wistar rats. The study employed wistar rats (150 - 250 g) which were administered carboxymethylcellulose 10 g/l (control), lead acetate solution (120 mg/kg), lead acetate solution (120 mg/kg) and succimer (10 mg/kg BW); lead acetate solution (120 mg/kg) and resveratrol (200 mg/kg); lead acetate solution (120 mg/kg) and resveratrol (400 mg/kg); and resveratrol alone (400 mg/kg) then administered lead acetate solution (120 mg/kg) daily for 2 weeks and considered as prophylactic group. All treatments were through the oral routes for different days. The acute toxicity of resveratrol was evaluated using the up and down method via oral routes in rats. The animal's body weights were recorded on days 1, 7 and 20. Also after animals were euthanized, relative organ weights were evaluated. Blood, plasma and organs samples were evaluated for blood lead levels (BLLs), heamatological analysis, biochemical analysis and histopathology. The LD_{50} was found to be above 5000 mg/kg. The results showed no significant ($p > 0.05$) change in body weight (BW) in resveratrol-treated group compared to the positive control group. Resveratrol-pretreated group showed improved BW compared to that of the positive control rats, although the difference was not significant ($p > 0.05$). There was significant ($p < 0.001$) decrease in BLLs of resveratrol-treated groups compared to both negative and positive control groups. No significant ($p > 0.05$) change was recorded for the liver function parameters

and electrolytes concentration, when the resveratrol-treated rats were compared to negative and positive control groups. There was significant ($p < 0.05$) increase in platelet counts in resveratrol-treated group (392.33 ± 31.81) compared to both negative (219.50 ± 30.50) and lead acetate treated group (210.50 ± 24.99). No significant ($p > 0.05$) change was recorded for the other heamatological parameters, when the resveratrol-treated groups were compared to negative and positive control groups. In the histopathology, the toxic effect of lead recorded in liver, kidney, heart and brain in the positive control group were significant ($p < 0.05$) when compared to resveratrol-treated groups. The toxic effects caused by lead were reduced to minimal level in resveratrol-treated groups. In conclusion, resveratrol ameliorated the adverse effects induced by lead in male wistar rats. This suggests that resveratrol may contain pharmacological compounds that could be useful in treatment of lead poisoning.

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LIST OF ABBREVIATIONS

ADHD Attention deficit hyperactivity disorder

ADP Adenosine diphosphate

ALA aminolevulinic acid

ALAD aminolevulinic acid dehydratase

ALKP Alkaline phosphatase

ALP alkaline phosphatase

ALT Alanine aminotransferase

AMP Adenine 3', 5'monophosphate

AMPK AMP-activated kinase

AST aspartate aminotransferase

ATM Ataxia telangiectasia-mutated

ATR Ataxia telangiectasia-Rad3-related

ATSDR Agency for toxic substance and disease registry

BAX Bcl-2-associated X protein

Bcl-2 B-cell lymphoma 2

BGT Black globe temperature

BLL Blood lead level

BMD Bone mineral density

BUN Blood urea nitrogen

CAT Catalase

CDC Centers for Disease Control and Prevention

Cl^- Chloride anion

CLIA Clinical Laboratory Improvement Amendments

CMC Carboxymethylcellulose

CNS Central nervous system

COX-2 Cyclooxygenase- 2

CPF chlorpyrifos

DBT Dry-bulb thermometer

DCP Disease control and prevention

DNA Deoxyribonucleic acid

ERK Extracellular signal-regulated kinase

GGT gamma-glutamyl transferase;

GPx Glutathione peroxidase

GSH glutathione LDH

H_2O_2 Hydrogen peroxide

HCO_3^{2-} bicarbonate

HepG2 Human hepatoblastoma cell line

HLI Heat load index

HOCl Hypochlorous acid

HPA Hypothalo-pituitary-adrenal

ICP-MS Inductively Coupled Plasma-Mass Spectrometry

K^+ potassium ion

LA lead acetate

LCII Lead Care II analyzer

LDH lactate dehydrogenase

MSF Médecins Sans Frontières

N/L Neutrophil/lymphocyte

Na^+ sodium ion

NADPH Nicotinamide adenine dinucleotide phosphate hydrogen

NIOSH National Institute of Occupational Safety and Health

NO Nitric oxide

O_2 Oxygen

PPAR-γ Peroxisome proliferator-activated receptor- γ

SIRT1 Sirtuin 1

SOD superoxide dismutase

TA taurine antioxidant

TNF Tissue necrotic factor

UNEP United Nations Environment Programme

WBC White blood cell

WHO World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Lead is a heavy, low melting, bluish-gray metal that occurs naturally in the Earth's crust. However, it is rarely found naturally as a metal. It is usually found combined with two or more other elements to form lead compounds. For these reasons, lead has been used by humans for *millennia* and is widespread today in products as diverse as pipes, storage batteries, pigments and paints, glazes, vinyl products, weights, shot and ammunition, cable covers, and radiation shielding. Tetra-ethyl lead was used extensively from the 1930s to the 1970s as a petrol additive to improve engine performance (Rosner and Markowitz, 1985; Landrigan *et al*., 2002). Tetra-ethyl lead has been eliminated from the petrol supplies of the majority of countries, but is still used in about nine countries (UNEP, 2008).

Lead poisoning (also known as *Plumbism, Colica Pictonum, aturnism*, Devon colic, or painter's colic) is a medical condition in humans and other vertebrates caused by increased levels of the heavy metal lead in the body (Washington, 2011). Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestines, kidneys, reproductive and nervous systems. It interferes with the development of the nervous system and is therefore particularly toxic to children, causing potentially permanent learning and behaviour disorders. Symptoms include abdominal pain, confusion, headache, anemia, irritability, and in severe cases seizures, coma, and death (Washington, 2011).

Routes of exposure to lead include contaminated air, water, soil, food, and consumer products. Occupational exposure is a common cause of lead poisoning in adults. According to estimates made by the National Institute of Occupational Safety and Health (NIOSH), more than three million workers in the United States are potentially exposed to lead in the workplace (Staudinger and Roth, 1998) . One of the largest threats to children is lead paint that exists in many homes, especially older ones; thus children in older housing with chipping paint or lead dust from moveable window frames with lead paint are at greater risk. Prevention of lead exposure can range from individual efforts (e.g. removing lead-containing items such as piping or blinds from the home) to nationwide policies (e.g. laws that ban lead in products, reduce allowable levels in water or soil, or provide for cleanup and mitigation of contaminated soil, etc.)

Elevated lead in the body can be detected by the presence of changes in blood cells visible with a microscope and dense lines in the bones of children seen on X-ray (Washington, 2011). However, the main tool for diagnosis is measurement of the blood lead level. When blood lead levels are recorded, the results indicate how much lead is circulating within the blood stream, not the amount being stored in the body (Washington, 2011). There are two units for reporting blood lead level, either micrograms per deciliter (µg/dl), or micrograms per 100 grams (µg/100 g) of whole blood, which are numerically equivalent. The US Centers for Disease Control (CDC) has set the standard for elevated blood lead level for adults to be 10 µg/dl of the whole blood. For children however, the number is set much lower at 5 µg/dl of the whole blood down from a previous 10 µg/dl (DCP, 2012). Children are especially prone to the health effects of lead and as a result, blood lead levels must be set lower and closely monitored for

contamination (Washington, 2011). The major treatments approaches are the removal of the source of lead and chelation therapy, administration of agents that bind lead so it can be excreted (Washington, 2011).

Humans have been mining and using this heavy metal for thousands of years, poisoning themselves in the process (CDC, 2012). Although lead mining is one of the oldest known occupation and a contributor to environmental hazards, the modern understanding of the small amount of lead necessary to cause harm did not come about until the latter half of the 20th century. No safe threshold for lead exposure has been suggested, that is there is no known amount of lead that is too small to cause body harm.

Resveratrol (3, 5, 4'-trihydroxystilbene) is a polyphenol that occurs naturally in foods and drinks made from grapes and peanuts, and also in a number of herbal remedies, both alone and as part of plant extracts. Resveratrol attracted little interest until 1992, when it was postulated to explain some of its cardioprotective properties and was thought to account in part for the so-called 'French Paradox', that is, the finding that the rate of coronary heart disease mortality in France is lower than that observed in other industrialized countries with a similar risk factor profile (Siemann and Creasy, 1992). Since then, reports have shown that resveratrol prevents or slows the progression of a wide variety of illnesses, including cancer, cardiovascular disease (Bradamante *et al.*, 2004) and ischaemic injuries (Sinha *et al.*, 2002).

Resveratrol enhances stress resistance and extends the lifespan of various organisms from yeast to vertebrates (Valenzano *et al*., 2006); it reduces the incidence of breast cancer (Whitsett and Lamartiniere, 2006; Wesierska-Gadek *et al*., 2007; Ferry-Dumazet

et al., 2002; Joe *et al*., 2002), cardiovascular diseases (Renaud and de- Lorgeril, 1992; Renaud and Gueguen, 1998), and possesses antioxidant property (Giovannini *et al*., 2001).

Resveratrol is a potent antioxidant, demonstrated to ameliorate adverse effects of heat stress-induced toxicity (Putics *et al*., 2008; Das, 2011, Sahin *et al*., 2012). Information on the ameliorative effect of resveratrol on heavy metals induced organ toxicity is scanty. The present study was undertaken to assess the ameliorative effect of resveratrol on lead induced organ toxicity in rats.

1.2 Statement of Research Problem

Although lead poisoning has been reported for centuries, it is a relatively new phenomenon in Nigeria. The recent unregulated mining activities in Zamfara State which resulted in serious toxicities and medical problems among adults and children alike is a source for concern. These unregulated mining activities are becoming more widespread in Nigeria and if unchecked, the medical and social consequence will be staggering.

Unfortunately, the people involved in this unregulated mining have little/no education and therefore lack the ability to protect themselves from the ensuing toxicities and medical problems. Reports have so far indicated that children are worse affected with little or no medical help coming their way (MSF, 2010; Blacksmith Institute, 2011). As from March - June 2010, 163 death cases of lead poisoning was reported (BBC News, 2010; independent News and Media, 2010) and 355 cases discovered (BBC News, 2010) with 46 % proving fatal (Yahaya and Sahabi, 2010). From May 2009 to May 2010, 25% (118 of 463) of children greater than 5 years of age in the surveyed compounds were

reported to have died and 82% (97 of 118) were reported to have had convulsion before death (Lo *et al*., 2010).

Effects of lead, such as inhibition of aminolevulinic acid dehydratase (ALAD), elevation of aminolevulinic acid (ALA) in urine and increase of free erythrocyte protoporphyrin in blood, have been observed in humans exposed to lead (Moore, 1988).

1.3 Justification

Lead poisoning is increasingly becoming a health problem with catastrophic consequences especially among children, whereas the known remedies are not easily available and affordable. The treatment for lead poisoning involves the use of chelating agents EthyleneDiamineTetraacetic Acid, Dimercaptosuccinic Acid, and Succimer (Graziano *et al.*, 1985; O'Connor and Rich, 1999) which may not readily be available for such affected rural communities to use.

This therefore necessitates the need for research into possible ethno medicinal forms of treatment that may be easily accessible and affordable. Resveratrol found in grapes, peanut and many other fruits and food items are indigenous and research have shown its therapeutic potentials in curing many medical problems and is reported to have central nervous system, antioxidant, chelating and anti-aging properties (Baur and Sinclair, 2006). These important properties make resveratrol an attractive candidate for investigation with respect to its potential as an antidote/chelating or ameliorative agent in lead poisoning.

1.4 Aim and Objectives of the Study

1.4.1 *Aim*

The main aim of this study is to evaluate the possible ameliorative effects of resveratrol on lead induced organ toxicity in male wistar rats.

1.4.2 *Objectives*

The objectives of this study are to:

- Assess effects of resveratrol on lead induced toxicities acute and sub-acute in wistar rats.
- Determine body and relative organ weight of wistar rats treated with resveratrol in presence of lead.
- Determine blood lead levels (BLLs) of wistar rats treated with resveratrol in presence of lead.
- Investigate the possible prophylactic effect of resveratrol in preventing lead-induced toxicity in wistar rats.
- Evaluate the effect of resveratrol on hematological, biochemical and histo-pathological parameters in lead-induced toxicity in wistar rats.

1.5 Statement of Research Hypothesis

Resveratrol ameliorates the adverse effects of lead induced organ toxicity in wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Lead Poisoning

Lead serves no useful purpose in the human body, but its presence in the body can lead to toxic effects, regardless of exposure pathway. Lead toxicity can affect every organ system. At the molecular level, proposed mechanisms for toxicity involve fundamental biochemical processes. These include lead's ability to inhibit or mimic the actions of calcium (which can affect calcium-dependent or related processes) and to interact with proteins (including those with sulfhydryl, amine, phosphate and carboxyl groups) (ATSDR, 2005).

It must be emphasized that there may be no threshold level for developmental effects in children. The practicing health care provider can distinguish overt clinical symptoms and health effects that come with high exposure levels on an individual basis. However, lack of overt symptoms does not mean "no lead poisoning." Lower levels of exposure have been shown to have many subtle health effects. Some researchers have suggested that lead continues to contribute significantly to socio-behavioral problems such as juvenile delinquency and violent crime (Needleman *et al*., 2002, Nevin, 2000). While the immediate health effect of concern in children is typically neurological, it is important to remember that childhood lead poisoning can lead to enduring health effects later in life including renal effects, hypertension, reproductive and developmental problems with their offspring.

2.2 Neurological Effects of Lead Poisoning

The nervous system is the most sensitive target of lead exposure. There may be no lower threshold for some of the adverse neurological effects of lead in children. Neurological effects of lead in children have been documented at exposure levels once thought to cause no harmful effects (< 10 µg/dL) (Canfield, 2003; CDC, 1997a). Because otherwise asymptomatic individuals may experience neurological effects from lead exposure, clinicians should have a high index of suspicion for lead exposure, especially in the case of children.

2.2.1 *Neurological Effects of Lead Poisoning in Children*

In children, acute exposure to very high levels of lead may produce encephalopathy and other accompanying signs of ataxia, hyperirritability, stupor, coma, convulsions and death. The blood lead levels (BLLs) associated with encephalopathy in children vary from study to study, but BLLs of 70-80 µg/dL or greater appear to indicate a serious risk (ATSDR, 2005). Even without encephalopathy symptoms, these levels are associated with increased incidence of lasting neurological and behavioral damage (ATSDR, 2005).

Children suffer neurological effects at much lower exposure levels. Neurological effects may begin at low BLLs, 10 µg/dL or lower, in some cases, and it may not be possible to detect them on clinical examination. Some studies have found, for example, that for every 10 µg/dL increase in BLL, children's IQ was found to be lower by four to seven points (Schroeder *et al.,* 1985; Fulton *et al.,* 1987; Landsdown *et al.,* 1986; Hawk *et al.,* 1986; Winneke *et al.,* 1990).

There is a large body of evidence that associates decrement in IQ performance and other neuropsychological defects with lead exposure. There is also evidence that attention deficit hyperactivity disorder (ADHD) and hearing impairment in children increase with increasing BLLs, and that lead exposure may disrupt balance and impair peripheral nerve function (ATSDR, 2005). Some of the neurological effects of lead in children may persist into adulthood. Toxic effects of exposure to lead in children and adults, affects at least three major organ systems: (1) the central and peripheral nervous systems; (2) the heme biosynthetic pathway; and (3) the renal system. Clinical manifestations differ somewhat between children and adults. In the child, the most serious symptoms are found in the central nervous system with subtle effects (e.g., decreased IQ and cognitive effects) occurring at lower levels and severe effects (e.g., seizures, encephalopathy) occurring at higher levels. Chelation therapy has reduced the mortality rate and morbidity substantially at higher levels. However, chelation therapy at lower levels (< 45 μg/dL), has not been shown to be as effective as removal of the lead source from the child's environment. Children are much more sensitive than adults to the neurocognitive and behavioral effects of lead, probably primarily for two reasons: (1) children absorb 40 to 50% of dietary lead whereas adults absorb about 10%; and (2) the young and developing nervous system is more vulnerable to chemical insult than the adult's. The blood lead threshold (if there is one) for neurocognitive and behavioral effects is probably lower in children than in adults (Bellinger *et al.*, 1992; Lanphear *et al*., 1995). Lead has been clearly demonstrated to produce tubular nephrotoxicity and chronic interstitial nephritis in humans and rodents after chronic exposure. Lead interferes in the formation of active vitamin D, which has an important role of influencing calcium metabolism. Calcium is

under tight homeostatic control in all cells (Onalaja and Claudio, 2000). The active form of Vitamin D is produced, primarily, from activation of Vitamin D by sunlight on the skin. The circulating hormone binds to Vitamin D Receptors (VDRs) in the nucleus of cells in the gastrointestinal tract, kidney and bone. This binding activates a cascade of events to increase calcium absorption. Because of their similar biochemical nature, lead can be absorbed by this mechanism especially in children who have decreased calcium intake. In addition, calbindin-D, the binding protein that aids in calcium transport, binds to lead with high affinity and may increase transport of lead in low calcium states (Onalaja and Claudio, 2000)

2.2.2 *Neurological Effects of Lead Poisoning in Adults*

There can be a difference in neurological effects between an adult exposed to lead and a child exposed lead when the brain was developing. Childhood neurological effects, including ADHD, may persist into adulthood. Lead-exposed adults may also experience many of the neurological symptoms experienced by children, although the thresholds for adults tend to be higher.

Lead encephalopathy may occur at extremely high BLLs, e.g., 460 µg/dL (Kehoe, 1961 and ATSDR, 2005). Precursors of encephalopathy, such as dullness, irritability, poor attention span, muscular tremor and loss of memory may occur at lower BLLs.

Less severe neurological and behavioural effects have been documented in lead-exposed workers with BLLs ranging from 40 to 120 µg/dL (ATSDR, 2005). These effects include decreased libido, depression/mood changes, headache, diminished cognitive performance, diminished hand dexterity, diminished reaction time, diminished visual motor performance, dizziness, fatigue, forgetfulness, impaired concentration, impotence

increased nervousness, irritability, lethargy, malaise, paresthesia, reduced IQ scores and lethargy. There is also some evidence that lead exposure may affect adults' postural balance and peripheral nerve function (ATSDR, 2007a, ATSDR, 2007b; Arnvig *et al*., 1980; Haenninen *et al*., 1979; Hogstedt *et al.*, 1983; Mantere *et al.*, 1982; Valciukas *et al.*, 1978, ATSDR, 1999). Slowed nerve conduction and fore-arm extensor weakness (wrist drop), as late signs of lead intoxication, are more classic signs in workers chronically exposed to high lead levels

2.3 Renal Effects of Lead Poisoning

Many studies show a strong association between lead exposure and renal effects (ATSDR, 1999). Acute high dose lead-induced impairment of proximal tubular function manifests in aminoaciduria, glycosuria and hyperphosphaturia (a Fanconi-like syndrome). These effects appear to be reversible (ATSDR, 1999). However, continued or repetitive exposures can cause a toxic stress on the kidney, if unrelieved; this may develop into chronic and often irreversible lead nephropathy (i.e., chronic interstitial nephritis). In children, lead appears to have an effect on renal function even at levels below 10 µg/dL (Loghman-Adham, 1998). This is especially true if the lead exposure occurs over a sustained period of time. Subtle abnormalities in renal tubular function, associated with aminoaciduria, glycosuria and increased excretion of low-molecular weight proteins can occur.

The lowest exposure level at which lead has an adverse effect on the kidney remains unknown. Most documented renal effects for occupational workers have been observed in acute high-dose exposures and high-to-moderate chronic exposures (BLL > 60 µg/dL). Currently, there are no early and sensitive indicators (e.g., biomarkers) considered

predictive or indicative of renal damage from lead (ATSDR, 2000). Serum creatinine and creatinine clearance are used as later indicators. However, certain urinary biomarkers of the proximal tubule (e.g., N-acetyl glycerol/NAG) show elevations with current exposures, even at BLLs less than 60 µg/dL; and some population-based studies show accelerated increases in serum creatinine or decrements in creatinine clearance at BLLs below 60 µg/dL (Staessen *et al*., 1992; Kim *et al*., 1996).

Latent effects of lead exposure that occurred years earlier in childhood may cause some chronic advanced renal disease or decrement in renal function. In children, the acute lead-induced renal effects appear reversible with recovery usually occurring within two months of treatment (Chisolm and Harrison, 1976). Treatment of acute lead nephropathy in children appears to prevent the progression to chronic interstitial nephritis (Weeden, 1992).

It should be noted that lead-induced end-stage renal disease is a relatively rare occurrence in the U.S. population. Renal disease can be asymptomatic until the late stages and may not be detected unless tests are performed. Because past or ongoing excessive lead exposure may also be a causative agent in kidney disease associated with essential hypertension (ATSDR, 1999), primary care providers should follow closely the renal function of patients with hypertension and a history of lead exposure.

Lead exposure is also believed to contribute to "saturnine gout," which may develop because of lead-induced hyperuricemia due to decreased renal excretion of uric acid. In one study, more than 50% of patients suffering from lead nephropathy also suffered from gout (ATSDR, 2000). Saturnine gout is characterized by less frequent attacks than primary gout. Lead-associated gout may occur in pre-menopausal women, an uncommon

occurrence in non-lead-associated gout (Goyer, 2001; ATSDR, 2000). A study by Batuman *et al*., (1989) suggests that renal disease is more frequent and more severe in saturnine gout than in primary gout.

2.4 Hematological Effects of Lead Poisoning

Lead inhibits the body's ability to make hemoglobin by interfering with several enzymatic steps in the heme pathway. Specifically, lead decreases heme biosynthesis by inhibiting *d*-aminolevulinic acid dehydratase (ALAD) and ferrochelatase activities. Ferrochelatase, which catalyzes the insertion of iron into protoporphyrin IX, is quite sensitive to lead. A decrease in the activity of this enzyme results in an increase of the substrate, erythrocyte protoporphyrin (EP), in the red blood cells (also found in the form of ZPP-bound to zinc rather than to iron). Also associated with lead exposure is an increase in blood and plasma d-aminolevulinic acid (ALA) and free erythrocyte protoporphyrins (FEP) (EPA, 1986; ATSDR, 1999).

Environmental Protection Agency (EPA) estimated the threshold BLL for a decrease in hemoglobin to be 50 µg/dL for occupationally lead exposed adults and approximately 40 µg/dL for children, although other studies have indicated a lower threshold (25 µg/dL) for children (EPA, 1986; ATSDR, 1999).

Recent data indicate that the erythrocyte protoporphyrin (EP) level, which has been used in the past to screen for lead toxicity, is not sufficiently sensitive at lower levels of blood lead and is therefore not as useful a screening test as previously thought.

Lead can induce two types of anemia, often accompanied by basophilic stippling of the erythrocytes (ATSDR, 1999). Acute high-level lead exposure has been associated with

hemolytic anemia. Frank anemia is not an early manifestation of lead exposure and is evident only when the BLL is significantly elevated for prolonged periods. In chronic lead exposure, lead induces anemia by both interfering with heme biosynthesis and by diminishing red blood cell survival. The anemia of lead intoxication is hypochromic and normo- or microcytic with associated reticulocytosis.

The heme synthesis pathway, on which lead has an effect, is involved in many other processes in the body including neural, renal, endocrine and hepatic pathways. There is a concern about the meaning of and possible sequelae of these biochemical and enzyme changes at lower levels of lead.

2.5 Endocrine Effects of Lead Poisoning

Studies in children with high lead exposure have shown that a strong inverse correlation exists between BLLs and vitamin D levels. Lead impedes vitamin D conversion into its hormonal form, 1, 25-dihydroxyvitamin D, which is largely responsible for the maintenance of extra- and intra-cellular calcium homeostasis. Diminished 1, 25-dihydroxyvitamin D in turn, may impair cell growth, maturation, and tooth and bone development. In general, these adverse effects seem to be restricted to children with chronically high BLLs (most striking in children with BLLs > 62 µg/dL) and chronic nutritional deficiency, especially with regard to calcium, phosphorous, and vitamin D (Koo *et al.*, 1991, ATSDR, 1999). However Rosen *et al*., (1980) noted that in lead-exposed children with blood lead levels of 33-55 µg/dL, 1, 25-dihydroxyvitamin D levels were reduced to levels comparable to those observed in children with severe renal insufficiency. Lead appears to have a minimal if any effect on thyroid function.

2.6 Gastrointestinal Effects of Lead Poisoning

In severe cases of lead poisoning, children or adults may present with severe cramping or abdominal pain, which may be mistaken for an acute appendicitis. Colic is a consistent early symptom of lead poisoning in occupationally exposed cases or in individuals acutely exposed to high levels of lead, such as occurs during the removal of lead-based paint. Colic is characterized by a combination of the following symptoms: abdominal pain, constipation, cramps, nausea, vomiting, anorexia and weight loss. Although gastrointestinal symptoms typically occur at PbBLs of 100–200 μg/dL, they have sometimes been noted in workers whose PbBLs were between 40 and 60 μg/dL (Pagliuca *et al*., 1990; Rosenman *et al*., 2003; Schneitzer *et al*., 1990). Colic is also a symptom of lead poisoning in children EPA (1986) has identified a LOAEL of approximately 60–100 μg/dL for children. This value apparently is based on a National Academy of Sciences (NAS, 1972) compilation of unpublished data from the patient groups originally discussed in Chisolm and Harrison (1976) in which other signs of acute lead poisoning, such as severe constipation, anorexia, and intermittent vomiting occurred at PbBLs ≥ 60 μg/dL.

2.7 Cardiovascular Effects of Lead Poisoning

Hypertension is a complex condition with many different causes and risk factors, including age, weight, diet, and exercise habits. Lead exposure is one factor of many that may contribute to the onset and development of hypertension. Although low to moderate lead level exposures (BLL<30 μg/dL) show only a low degree of association with hypertension, higher exposures (primarily occupational) increase the risk for hypertensive heart disease and cerebrovascular disease as latent effects. One study found that adults

who experienced lead poisoning in childhood had a significantly higher risk of developing hypertension 50 years later relative to control adults without childhood lead exposure (Hu, 1991; ATSDR, 2000). The association has been shown in population-based studies with BLLs below 10 µg/dL. Data supports an association between lead exposure and elevations in blood pressure (Victery *et al;* 1982; ATSDR 2000; Korrick *et al.* 1999; Hu *et al.* 1996).

It is estimated that, on a population basis, blood lead can account for a 1 to 2 % variance in blood pressure (ATSDR, 2000). This could increase the incidence of hypertension substantially due to the high prevalence of hypertension of all causes in the general population.

2.8 Reproductive Effects of Lead Poisoning

Reproductive effects examined in the literature include sperm count, fertility, and pregnancy outcomes. While several studies have implicated lead as contributing to reproductive and developmental effects, these effects have not been well-established at low exposure levels (ATSDR, 2000).

2.8.1 *Male Reproductive Effects in Lead Poisoning*

Studies on reproductive function studies in humans suggest that current occupational exposures decrease sperm count totals and increase abnormal sperm frequencies (ATSDR, 2000). Effects may begin at BLLs of 40 µg/dL (ATSDR, 2005) Long-term lead exposure (independent of current lead exposure levels) also may diminish sperm concentrations, total sperm counts, and total sperm motility (ATSDR 2000). It is unclear how long these effects may last in humans after lead exposure ceases. It is not currently

possible to predict fertility outcomes based on current BLLs or past lead exposure levels (ATSDR, 2000).

2.8.2 *Pregnancy Outcomes in Lead Poisoning*

The effect of low-level lead exposures on pregnancy outcomes is not clear. Thus it appears that at higher (*e.g.*, occupational) exposure levels, the evidence is clearer for an association between lead and adverse pregnancy outcomes. This association becomes equivocal when looking at women exposed to lower environmental levels of lead. The data concerning exposure levels are incomplete, probably as a result of far greater exposures than are currently found in lead industries. Some studies in women living near smelters versus those living some distance away did show increased frequency of spontaneous abortions (Nordstrom *et al.,* 1979) and miscarriages and stillbirths (Baghurst *et al.,* 1987; McMichael *et al.,* 1986). In contrast Murphy *et al.,* (1990) evaluated past pregnancy outcomes among women living in the vicinity of a lead smelter and did not find an increase in spontaneous abortion risk among the lead exposed group versus the unexposed group.

Women with BLL 5-9 µg/dL were two to three times more likely to have a spontaneous abortion than were women with BLL lesser than 5 µg/dL (Borja-Aburto *et al.,* 1999). The reproductive toxicity which results from high dose lead exposure was well known in the last century. In fact the data of the latter half of the 1800s led a British royal commission to recommend in 1910 that women not be employed in the lead trades. This has only changed in the last 30 years, with the return of women to the work force. The obvious effects of lead in the 19th century were stillbirth and spontaneous abortion, which was usually recognized in women with occupational exposure to lead (Borja-

Aburto *et al.,* 1999). In general, spontaneous abortion was an early event. At the present time, we do not know the lowest blood lead at which this may occur, because lead apparently has an effect on the implantation of the fertilized ovum in the uterus. With the advent of human chorionic gonadotropin measurement procedures, it is now possible to detect the onset of pregnancy and early fetal loss as early as the first one to two weeks of pregnancy (Borja-Aburto *et al.,* 1999). Sexual dysfunction in the male has not been as closely studied. The studies that have been published, which suggest hypospermia and teratospermia, for example, have been criticized for faulty design. More recently, it has been found in workers employed for more than three years that serum testosterone and free testosterone indices are decreased, at mean blood lead concentrations in excess of 60μg/dL.

2.9 Developmental Effects of Lead Poisoning

Developmental effects examined in the literature include pregnancy outcomes (*e.g.*, premature births and low birth weights), congenital abnormalities, and post birth effects on growth or neurological development. Increasing evidence indicates that lead, which readily crosses the placenta, adversely affects fetus viability as well as fetal and early childhood development. Prenatal exposure to low lead levels (*e.g.*, maternal BLLs of 14 μg/dL) may increase the risk of reduced birth weight and premature birth (ATSDR, 1999). Although lead is an animal teratogen, most human studies have not shown a relationship between lead levels and congenital malformations. A study by Needleman *et al.* (1984) correlated increased prenatal lead exposure with increased risk for minor congenital abnormalities (*e.g.*, minor skin abnormalities and undescended testicles). No

association between prenatal lead exposure and major congenital abnormalities has been found (McMichael *et al.,* 1986).

In a retrospective study, a higher proportion of learning disabilities were found among school-aged children whose biological parents were lead poisoned during childhood 50 years earlier (Hu, 1991).

2.10 Other Potential Effects of Lead Poisoning

Lead has been linked to problems with the development and health of bones. At high levels, lead can result in slowed growth in children. Studies have shown increased likelihood of osteoporosis (weakened bones later in life) in animals exposed to lead (ATSDR, 1999). Although this link has not been established in humans, it is likely that upon closer examination of lead-exposed individuals, lead will be shown to be a new risk factor for the disease.

Current available data are not sufficient to determine the carcinogenicity of lead in humans. Environmental Protection Agency (EPA) has classified elemental lead and inorganic lead compounds as Group 2B: probable human carcinogens (ATSDR, 1999). This classification is based in part on animal studies, which have been criticized because the doses of lead administered were extremely high (ATSDR, 1999). The National Toxicology Program classifies lead and lead compounds as "reasonably anticipated to be a carcinogen" (NTP, 2005). Information regarding the association of occupational exposure to lead with increased cancer risk is generally limited. This is because these occupational exposure studies, which primarily examined lead smelters, involved

confounding exposures to other chemicals, including arsenic, cadmium, antimony, and toxicants from worker smoking habits (Cooper, 1988; IARC, 2004).

2.11 Resveratrol

Resveratrol (3,5,4′-trihydroxystilbene; was first isolated from the roots of white hellebore (*Veratrum grandiflorum* O. Loes) in 1940 (Takaoka, 1940), and later in 1963 from the roots of *Polygonum cuspidatum*, a plant used in traditional Chinese and Japanese medicine (Nonomura *et al*., 1963). Initially characterized as a phytoalexin, resveratrol attracted little interest until 1992, when it was postulated to exhibit some cardioprotective effects of red wine (Sieman and Creasy, 1992). Since then, dozens of reports have established that resveratrol prevents or slows the progression of a wide variety of illnesses (Renaud and de- Lorgeril, 1992; Renaud and Gueguen, 1998; Sinha *et al.*, 2002; Ferry-Dumazet *et al*., 2002; Bradamante *et al.*, 2004; Valenzano *et al*., 2006; Wesierska-Gadek *et al*., 2007).

2.12 Sources of Resveratrol

The richest source of resveratrol is the roots of *Polygonum cuspidatum*, mainly cultivated in China and Japan. The skins of grapes contain about 50–100 mg of resveratrol, and they are believed to be responsible for the cardioprotective properties of red wine, which contains about 0.2–7 mg/L of wine. In addition to grapes, resveratrol is found in a large variety of fruits, including mulberry, bilberry, lingonberry, sparkleberry, deerberry, partridgeberry, cranberry, blueberry, jackfruit and peanut. A wide variety of flowers and leaves, including gnetum, white hellebore, corn lily, butterfly orchid tree, eucalyptus, spruce, poaceae, scots pine, and rheum also contain resveratrol. Resveratrol is synthesized in response to environmental stressors that include water deprivation, UV

irradiation, and especially, fungal infection. Thus, the production of resveratrol in plants may be considered as part of their defense mechanisms (Dipak and Nilanjana, 2006).

2.13 Chemical Structure of Resveratrol

Resveratrol (3, 5, 4'-trihydroxystilbene) is also called 5-[(E)-2(4-hydroxyphenyl)-ethenyl] benzene-1,3-diol or $C_{14}H_{12}O_{13}$ (Markus and Morris, 2008; Athar *et al*., 2009). The chemical structure of resveratrol is important because from its structure, information regarding its biological activity may be obtained. Due to the presence of more than one phenol groups, this agent is classified as a polyphenol. Polyphenols are often antioxidants as they can react with free radicals to form a stable molecule that is less toxic than the original radical (Aziz *et al*., 2003).

Resveratrol is found in both *trans*- and *cis*-forms (Fig. 2.1) with trans-resveratrol having the greater biological activity of the two (Baur and Sinclair, 2006; Shankar *et al*., 2007). Resveratrol in solution shows photosensitivity, and *trans*- to *cis*-isomerisation is facilitated by ultra-violet exposure (De la Lastra and Villegas, 2007). When protected from light, trans-resveratrol is stable for months, except in high-pH buffers, and *cis*-resveratrol is stable only near neutral pH. Besides, trans-resveratrol is not particularly sensitive to UV/fluorescent light, elevated relative humidity and ambient temperature, or atmospheric oxidants at ambient concentrations (Santos *et al*., 2011).

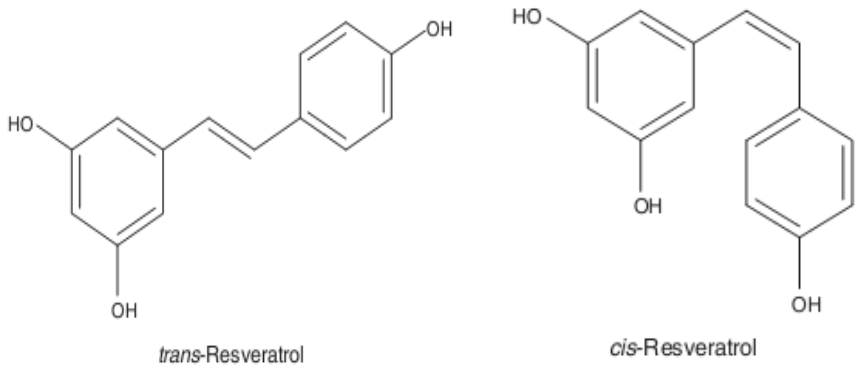


Figure 2.1: Chemical structure of *trans* and *cis*-resveratrol

2.14 Pharmacokinetics of Resveratrol

Oral intake is the major route of resveratrol administration. Since resveratrol is a lipophilic molecule, early administrations were performed by dissolving the pure compound in various alcoholic solutions such as white wine (Soleas *et al*., 2001; Charles-Henry *et al*., 2010), whisky, diluted ethanol (Walle *et al*., 2004), or in non-alcoholic liquids such as fruit or vegetable juices (Meng *et al*., 2004). Absorption of trans-resveratrol is significantly delayed by the presence of food (Vaz-da-silva *et al*., 2008). Therapeutic potentials of resveratrol have been achieved by administration of large doses that is not feasible through dietary intake. Red wine seems to be the richest source of resveratrol in human diet, and the richest red wine contains resveratrol at the concentration of 5-7 mg/L (Gescher and Steward, 2003). Thus, in order to achieve the dose of resveratrol fed to the fish and mice (Baur *et al*., 2006; Valenzano *et al*., 2006), pharmaceutical means only must be employed, and not through dietary means (Brown *et al*., 2009).

2.14.1 *Absorption*

From animal studies and from limited human studies, resveratrol is apparently absorbed from the gastro-intestinal tract following its ingestion (Shankar *et al*., 2007). Polyphenols, unlike resveratrol, are only partially absorbed, and only a fraction is excreted via the urine in humans. Although plasma concentrations achieved and the urinary yield, as a function of oral dose, depend on a number of factors, by far the most important is the structure of the polyphenol (Brown *et al*., 2009).

2.14.2 *Metabolism*

Polyphenols are extensively metabolized during absorption from the gut, and the typical products are phase-2 conjugates (that is, glucuronidated, sulphated and methylated derivatives) of the parent polyphenol. *Trans*-resveratrol is the isomer most used in human pharmacological studies because not much is known about *cis*-resveratrol in comparison, for reasons of lack of stability (Santos *et al*., 2011). Studies on resveratrol have been done mostly *in vitro*, but little have been done *in vivo* in mammals like mice and pigs, and results have shown that resveratrol possesses pharmacological activities in the mammalian gut. It is not known whether dietary resveratrol in humans will reach the multiple proposed sites of actions beyond the gastro-intestinal tract. To address this issue, a study was conducted by Walle *et al*., (2004) to observe the absorption and bioavailability of resveratrol after oral and intravenous doses in six human volunteers. The absorption of 25 mg oral dose of resveratrol was 70%, with peak plasma levels of resveratrol and metabolites of 491 ± 90 ng/ml and plasma half-life of 9.2 ± 0.6 h, and only trace amounts of unchanged resveratrol (less than 5 ng/ml) was detected in the plasma. It was also observed that the oral dose was recovered in urine (53.4%-84.9%),

the recovery in faeces was 0.3-38.1%. After intravenous dose, 42.3-83.2% was recovered in urine and 0.6-22.7% was found in faeces. Total recovery in urine and faeces were 70.5-97.6% after oral dose and 53.5-91.2% following intravenous dose (Walle *et al*., 2004).

Liquid chromatography/mass spectrometry analysis identified three metabolic pathways, that is, sulphate, glucuronic acid conjugation of the phenolic groups and hydrogenation of the aliphatic double bond, likely produced by intestinal microflora. Five distinct metabolites indicated in the earlier study were; resveratrol monoglucunoride, isomeric resveratrol monoglucunoride, dihydroresveratrol monoglucunoride, resveratrol monosulphate, and dihydroresveratrol sulphate. Amongst them the sulphate conjugates were clearly detected in plasma within 2 hours after oral or intravenous doses. In another study by Brown *et al.,* (2010), an additional metabolite (resveratrol sulphate glucuronides) was detected by high performance liquid chromatography/ultraviolet. According to these authors, the mean plasma concentration and maximum values of parent resveratrol across the four dose levels ranged from 0.04 to 0.55 nmol/mL, and 0.19 to 4.24 nmol/mL, respectively, with resveratrol-3-O-sulphate having the highest plasma concentration. The plasma elimination half-lives varied between 4.77 and 9.70 hours for resveratrol and between 3.09 and 8.14 hours for the major metabolites. Thus, the study also confirmed the rapid metabolism and low bioavailability of resveratrol. An earlier study by Niles *et al*., (2006) on tumour-bearing mice observed that, 5 min after abolus gavage of 75 mg/kg resveratrol, plasma concentrations of resveratrol were 28.4 ± 36.4 μmol/L. Two major metabolites of resveratrol were also detected in plasma: resveratrol glucuronide, which was present at higher amounts than resveratrol; and piceatannol,

present at lower amounts than resveratrol. *In vitro* metabolic studies in rat liver microsomes using LC/MSMS and LX/EC also found piceatannol (Zhu *et al*., 2003) as a major metabolite of resveratrol. The liver contained higher amounts of resveratrol than either the skin or plasma. However, in the plasma and liver, the major metabolite, resveratrol glucuronide, was present at equivalent or higher concentrations than resveratrol, whereas in the skin, resveratrol glucuronide concentration was considerably lower than resveratrol (Zhu *et al*., 2003).

The pivotal role the liver plays in the bioavailability of resveratrol makes it a rate-limiting step in the distribution of resveratrol to other tissues in the body (Charles-Henry *et al*., 2010). Lancon *et al.,* (2007) explored the extensive metabolism of resveratrol in the human liver using human liver-derived HepG2 cells. Results of the experiments showed that the proportion of conjugated resveratrol was 20 % after 2 hours of incubation, and it rose to 50 % at 4 hours, and attained almost 100 % at 8 hours. The metabolites detected were also glucuronides and sulphate derivatives.

To by-pass the problem of resveratrol metabolism by the liver, Niles *et al*., (2006) implanted slow-release resveratrol-containing pellets next to newly developing tumours. This method of delivering resveratrol did not inhibit tumour growth. Since glucuronidation is known to reduce bioavailability of resveratrol, flavonoids have been demonstrated to inhibit resveratrol glucuronidation, and such an inhibition may improve the bioavailability of resveratrol (De Santi *et al*., 2000).

2.15 Safety of Resveratrol

To test the potential toxicity of resveratrol, rats were administered orally with 0, 300, 1000, and 3000 mg trans-resveratrol per kilogram body weight per day for four weeks

(Crowell *et al.*, 2004). Most of the adverse effects occurred in the rats administered 3000 mg per kilogram body weight per day. They included an increase in clinical signs of toxicity; reduced final body weights and food consumption; elevated blood urea nitrogen (BUN)**,** creatinine, alkaline phosphatase, alanine aminotransferase, total bilirubin, and albumin; reduced haemoglobin, haematocrit, and red blood cell counts; and increased white blood cell counts. Clinical chemistry changes; that is, increased alanine aminotransferase (ALT**),** alkaline phospatase (ALP**)**, and total bilirubin in the 3000 mg/kg body weight/day dose group, suggest liver toxicity, but this was not supported histologically. From the results obtained in the study the no-observed-adverse-effect level was 300 mg resveratrol per kilogram body weight per day in rats (Crowell *et al*., 2004). In another investigation by Juan *et al*., (2002), 20 mg resveratrol was administered orally per kilogram body weight to rats for 28 days. No treatment-related effects were reported, except mild changes in serum liver enzymes. Observation by Juan *et al*., (2005) of rats administered with 20mg/kg of resveratrol for 90 days showed unaccompanied side-effects, while studying the effect of resveratrol on sperm output. Clinical trials in healthy humans, volunteers administered with resveratrol at 0.5, 1.0, 2.5 or 5.0 g daily for 29 days reported resveratrol to be safe, as borne out by the lack of serious adverse reactions detected by clinical, biochemical or haematological analyses during the study. However, the 2.5 and 5.0 g doses caused mild to moderate gastro-intestinal symptoms (Brown *et al*., 2010). There was no weight loss in any participant, and all volunteers maintained normal performance status throughout the study period (Brown *et al*., 2010).

2.16 Measurement of Resveratrol

Although resveratrol exists naturally as both *cis*- and *trans*-isomers, most studies have used *trans*-resveratrol for administration due to lack of stability of the *cis*-isomer, which is not commercially available. For this reason, *trans*-resveratrol has often been reported to be the major natural form, although the *cis*- isomer is also present in wine (Soleas *et al*., 2001).

2.17 Pharmacological Potentials of Resveratrol

2.17.1 *Cardioprotective Effects*

The heart is an aerobic organ, and the energy required for its function is derived from oxidative phosphorylation. Oxidative stress caused by free radicals plays a crucial role in the pathophysiology associated with atherosclerosis, neoplasia and neurodegenerative diseases. The cardioprotective property of resveratrol is multidimensional. Resveratrol, at a very low concentration, inhibits apoptotic cell death, thereby providing protection from various diseases, including myocardial ischaemic reperfusion injury, atherosclerosis and ventricular arrhythmias (Wu *et al*., 2001; Thirunavukkarasu *et al*., 2007; Ungvari *et al*., 2007; Zhao *et al*., 2008). Both in acute and chronic models, resveratrol-mediated cardioprotection are achieved through the pre-conditioning, rather than direct effect as found in conventional medicine (Dipak and Nilanjana, 2006). The same resveratrol, when used in higher doses, facilitates apoptotic cell death, and behaves as a chemo-preventive alternative. Resveratrol likely fulfills the definition of a pharmacological pre-conditioning compound and gives hope for the therapeutic promise of alternative medicine (Das and Das, 2007).

2.17.2 *Resveratrol and Diabetes Mellitus*

Resveratrol is a natural polyphenolic compound that activates nicotinamide adenosine dinucleotide-dependent deacetylase sirtuins (SIRT1). It has recently been shown to exert potent antidiabetic actions, when orally delivered to animal models of type-2 diabetes (Ramadori *et al*., 2009). Intracerebro-ventricular infusion of resveratrol normalized hyperglycaemia and greatly improved hyperinsulinaemia in diet-induced obese and diabetic mice, and these effects were independent of changes in body weight, food intake and circulating leptin levels (Ramadori *et al*., 2009). In a study by Minikawa *et al*., (2011), resveratrol significantly suppressed the elevation in the fasting blood glucose, serum triglyceride and lipid peroxide levels in both type-2 diabetic mice and cell culture systems. Resveratrol stimulated glucose uptake and glucose transporter-4 translocation by activating both insulin signaling and AMP-activated protein kinase signaling. Besides, resveratrol may protect pancreatic β-cells from advanced glycation end-product-induced oxidative stress and apoptosis. These results established resveratrol anti-diabetic effect, by stimulating both insulin-dependent and independent glucose uptake in muscles and by protecting pancreatic β-cells from advanced glycation end-products-induced oxidative stress and apoptosis. Lagouge *et al*., (2006), Baur *et al*., (2006) and Sharma *et al*., (2005) also showed that resveratrol increased insulin sensitivity in high caloric diet, fed mice by AMPK-dependent mechanism

2.17.3 *Chondroprotection and Cognition in Ageing*

Osteoarthritis is a highly age-related inflammatory process of joints. When chondrocytes were pre-treated with various concentrations of resveratrol, the advanced glycation end products-induced expression of iNOS was suppressed, and resveratrol also inhibited

advanced glycation end-product-induced cyclo-oxygenase 2 (COX-2) expression. The results showed that resveratrol inhibited advanced glycation end-products-induced NO and PGE2 production (Liu *et al*., 2010). Resveratrol has been reported to preserve neuronal health and cognitive function in ageing mice by delaying age-dependent cerebrovascular deterioration (Oomen *et al*., 2009).

2.17.4 *Gastro-Intestinal Effect of Resveratrol*

Hepatic injury caused by ischaemia/reperfusion has been proposed as a key clinical problem, associated with liver transplantation and major liver surgery. Furthermore, hepatic ischaemia/reperfusion injury also occurs in diverse situations, such as heart failure, liver trauma, and blood occlusion to the liver. The production of reactive oxygen species has been demonstrated in reperfusion injury and resveratrol exerts several biologic effects, including a potent antioxidant effect *via* prevention of lipid peroxidation (Tadolini *et al*., 2000; Miura *et al*., 2003). Investigation by Gedik *et al*., (2008) suggested that resveratrol has protective effects against hepatic ischaemia/reperfusion injury, and is a potential therapeutic drug for ischaemia reperfusion-related liver injury.

2.17.5 *Platelet Aggregation and Resveratrol*

Blood platelets are multi-responding cells. They can be activated by several physiologically important compounds, including collagen, ADP, and thrombin, and are involved in blood clotting, inflammation process, atherogenesis, and cancers (Malinowska and Olas, 2011). Blood platelet aggregation under physiological conditions is an important process that arrests bleeding, but excessive platelet aggregation causes thrombosis and atherosclerosis (Olas and Wachowicz, 2005; Malinowska and Olas, 2011). Resveratrol was demonstrated to significantly reduce platelet aggregation and

superoxide anion radical production in platelets in a model of hyperhomocysteinaemia (Malinowska and Olas, 2011).

2.17.6 *Resveratrol and Cancer*

Probably the most studied properties of resveratrol, besides that of cardioprotection and life extension, are its anticancer properties. This is based on its ability to suppress proliferation of a wide variety of tumor cells, including lymphoid and myeloid cancers (Opipari *et al*., 2004), cancers of the breast (Tyagi *et al*., 2005), prostate (Zhou *et al*., 2005), stomach, colon, oesophageal carcinoma, pancreatic cancer (Kuwajerwala *et al*., 2002), melanoma (Tsan *et al*., 2002), endometrial cancer (Ding and Adrian, 2002), gastric carcinoma cells, (Kaneuchi *et al*., 2003) ovarian carcinoma (Liu *et al*., 2004) and cervical carcinoma (Zhou *et al*., 2003).

Telomerase activity, is absent in normal cells except germ cells and renewal tissues, is found in cancer cells (Kim, 1994). Sreenivasulu and Vijayalakshmi (2010) found resveratrol to inhibit telomerase activity, proffering this to be one of the mechanisms of resveratrol's anti-cancer properties. Investigation by Zhou *et al.*, (2005) on anticancer activity of resveratrol on implanted human primary gastric carcinoma cells in nude mice, using 500, 1000, 1500 mg/kg resveratrol directly injected beside tumour body 6 times at an interval of 2 days, established that resveratrol induced apoptosis of transplanted tumour cells. This apoptosis was suggested to be as a result of down-regulating apoptosis regulated gene bcl-2 and up-regulating the expression of apoptosis-regulated gene bax. Studies have also revealed that resveratrol apoptotic and anti-proliferative effects may result from its ability to induce prostate cancer cell entry into S phase. This increases its proliferative ability, making the cancer cells more sensitive to cytotoxic effects of

chemotherapeutic agents, and effectively inhibits DNA synthesis in both androgen-sensitive and androgen-independent prostate cancer cells, thus delaying the progression of prostate cancer (Kuwajerwala *et al*., 2002). Resveratrol enhances proliferation in low doses (0.1-1.0 μg/ml), and in high doses (10.0-100.0 μg/ml) it induces apoptosis and decreases mitotic activity (Szendel *et al.*, 2000).

Results by Tyagi *et al*., (2005) suggested that activation of ATM/ATR check-point as a central mechanism of resveratrol-induced S phase arrest and growth inhibition in ovarian cancer cells. Result of the studies also suggest that the anticancer effects of resveratrol may be mediated directly through COX-2 (Zykova *et al*., 2008), which is highly expressed in various cancers. Resveratrol directly binds with COX-2, and this binding is absolutely required for resveratrol's inhibition of the ability of human colon adenocarcinoma HT-29 cells to form colonies in soft agar. There are several other natural derivatives of resveratrol that are structurally similar to resveratrol, and are also present in food that are being explored for chemopreventive and anti- tumour properties (Fulda, 2010).

2.17.7 *Resveratrol and the Liver*

Studies carried on alcoholic fatty liver in mice concluded that resveratrol treatment reduced lipid synthesis and increased rates of fatty acid oxidation and prevented alcoholic liver steatosis. The protective action of resveratrol is in whole or in part mediated through the up-regulation of a SIRT1-AMPK signaling system in the livers of ethanol-fed mice. The results suggest that resveratrol may serve as a promising agent for preventing or treating human alcoholic fatty liver disease (Ajmo *et al*., 2008). Investigation by Shang *et*

al., (2008) on non-alcoholic fatty liver disease in rats also demonstrated that resveratrol had protective effect via activation of AMP-activated protein kinase.

2.17.8 *Neuroprotection by Resveratrol*

Oxidative stress has been implicated as a common underlying risk factor for the pathogenesis of many neurological, particularly neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease, spinal cord injury and stroke (Gilgun-Sherki, 2001; Yang and Piao, 2002; Sun *et al*., 2010). Many studies have demonstrated resveratrol's potential in the neuroprotection of neurodegenerative diseases (Virgili and Contestabile, 2000; Savaskan *et al*., 2003; Marambaud *et al*., 2005; Vingtdeux *et al*.,2008).The brain is exposed throughout life to excitatory amino acids (such as glutamate), whose metabolism generates ROS. The exposure promotes excitotoxicity and renders the brain susceptible to rapid neurodegeneration. Therefore, administration of dietary antioxidants to individuals (Esposito *et al*., 2002; Ates, 2006) may ameliorate the adverse effects of ROS, especially those that can cross the blood brain barrier. Resveratrol has also been shown to prevent spatial memory deficit, induced by intracerebro-ventricular administration of streptozotocin (Sharma *et al*., 2005). It alleviated lipopolysaccharide-induced neurotoxicity by a mechanism that may involve iron shuttling proteins in rats, pre-treated with resveratrol at a dose of 20 mg/kg for 7 days and challenged with a single dose of lipopolysaccharides, (Sebai *et al*., 2009). Studies by Virgili and Contestabile (2000) and Mokni *et al*. (2007) have also shown that resveratrol crosses blood-brain barrier and induces antioxidant enzyme activities. A protein, peroxisome proliferator-activated receptor-γ (PPAR-γ), has been proposed as a

therapeutic target for neurodegenerative disease due to its ability to protect against mitochondrial damage through up regulation of Bcl-2, an anti-apoptotic protein.

Resveratrol's ability to diminish amyloid plaques was tested using mice fed clinically feasible dosages of resveratrol for 45 days. Neither resveratrol nor its conjugated metabolites were detectable in brain, yet resveratrol diminished plaque formation in a region specific manner (Karuppagounder *et al*., 2009). Resveratrol has been demonstrated to protect the neonatal brain against hypoxic-ischaemic injury (West *et al*., 2007). It has also been demonstrated that resveratrol protects the brain against ischaemic stroke in mice through activation of the anti-apoptotic protein, Bcl-2 (Inoue *et al*., 2003).

2.17.9 *Antioxidant Activity of Resveratrol*

Resveratrol has been shown to exert antioxidant activity via free-radical scavenging, up regulation of natural antioxidant defences of cells (Kutuka *et al*., 2004; Ara *et al*., 2005; Ungvari *et al*., 2007; Kedzierska *et al*., 2011). The strong antioxidant properties of resveratrol have been associated with its chemical structure, which contains more than one phenol groups (polyphenol). The phenol groups react with free radicals to form a stable molecule that is less toxic than the original radical (Aziz *et al*., 2003). Resveratrol prevents low-density lipoprotein (LDL) oxidation *in vitro* by chelating copper, as well as by directly scavenging free radicals (Zou *et al*., 1999; Zou *et al*., 2000). Oxidative stress-induced DNA damages have also been demonstrated to be protected by resveratrol (Liu and Zheng, 2002). Cavallaro *et al*. (2003) investigated the effect of resveratrol on some activities of polymorphonuclear leucocytes, particularly the generation of superoxide anion (O_2^-) in whole blood, hypochlorous acid (HOCl) and nitric oxide (NO) production

by isolated cells. Resveratrol showed significant dose-dependent inhibitory effect on all these activities.

2.17.10 *Central Nervous System Activity of Resveratrol*

The central nervous system has been indicated to be a target of resveratrol. Phosphorylation of mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinase 1 (ERK1) and extracellular signal-regulated kinase 2 (ERK2) were induced by resveratrol on undifferentiated and differentiated SH-SY5Y human neuroblastoma cells at a dose of 1μmol, demonstrating that resveratrol, even at very low concentrations, may have a biological effect on neurone-like cells (Miloso *et al*., 1999). Zhuang *et al*. (2003) suggestedthat neuroprotection of resveratrol may be as a result of generation of haeme oxygenase which helps in the rapid degeneration of pro-oxidant haeme (iron-protoporphyrin IX).

Resveratrol also activates peroxisome proliferator-activator receptor, thus protecting the brain against ischaemia (Inoue *et al*., 2003; Tsukamoto *et al*., 2010). Stimulation of AMP kinase activities in neurones by resveratrol has also been reported (Dasgupta and Milbrandt, 2007).

2.17.11 *Resveratrol and Life Extension*

In recent years, there is considerable interest concerning investigation of antioxidative and anti- inflammatory effects of phenolic compounds including resveratrol, from different botanical sources. The mechanisms by which resveratrol prolongs life-span in model organism are not clear, but the observation that its supplementation with food extends vertebrate lifespan and delays motor and cognitive age-related decline may be of high relevance for the prevention of age-related diseases in the human population

(Valenzano *et al*., 2006). Among all methods suggested to extend lifespan, dietary restriction seems to be the most explored, which is simply reduction in caloric intake without malnutrition (Baur, 2010). Studies have shown that resveratrol extends lifespan by mimicking caloric restriction via activation of sirtuins [homologues of silent information regulator 2 (Sir 2) found in yeast]. This ability of resveratrol has been evidenced by studies on yeast (*Saccharomyces cerevisiae),* fruitfly *(Drosophila melanogaster),* nematode worm *(Caenorhabditis elegans),* and the short-lived fish *(Nothobranchius furzeri).* In each case, resveratrol increased the lifespan by 70%, 29%, 18% and 56%, respectively (Howitz *et al*., 2003; Bauer *et al*., 2004; Wood *et al*., 2004; Viswanathan *et al*., 2005; Valenzano *et al*., 2006). Some reports have challenged these findings. Bass *et al*. (2007), for instance, were unable to replicate the results in *C. elegans* and *Drosophila*, after seven independent trials. In *Drosophila*, no significant increase in life extension was recorded, and the results obtained from *C. elegans* were also variable, with resveratrol treatment resulting in slight increase in lifespan in some trials, but not others. The findings questioned the ability of resveratrol to extend life. Hope was restored in resveratrol's ability, when the effect of resveratrol in mammals, a mouse model of obesity, was reported by two separate groups in France (Lagouge *et al*., 2006), USA (Baur *et al*., 2006) and also by another group, whose results further substantiated the fact that resveratrol extends life by mimicking caloric restriction (Barger *et al*., 2008). Insulin signalling pathway has also been strongly linked to ageing process, and resveratrol has been suggested to extend life by inhibition of insulin signaling pathway, independent of its activation of SirT1 histone deacetylase (Zhang, 2006).

2.17.12 *Resveratrol and Obesity*

The number of overweight individuals worldwide has reached 2.1 billion, leading to an explosion of obesity-related health problems associated with increased morbidity and mortality (Li *et al*., 2005). Obesity stems from a prolonged imbalance between the level of energy intake and energy expenditure. It results in the storage of surplus as lipids, predominantly in adipose tissue, but also in muscle and liver tissues. Obesity triggers features of the metabolic syndrome (Dal-Pan *et al*., 2010), including hyperinsulinaemia, hypertension, dyslipidaemia and abdominal obesity, and is probably triggered by initial imbalances at the cellular level in various critical metabolic pathways. The cellular imbalance is characterized by an increase in the number and size of adipocytes (Virmania *et al*., 2007). Resveratrol has been shown to shift the physiology of middle-aged mice on a high-calorie diet towards that of mice on a standard diet as indicated by a variety of measures, including survival, motor function, insulin sensitivity, organ pathology, PGC-1a activity, and mitochondrial number. Notably, all the changes occurred without a significant reduction in body weight (Baur *et al*., 2006).

Chen (2008) also reported that resveratrol reduced body weight gain, fat tissue depositions and leptin levels in female mice, fed high-fat diet during the 16-week animal studies. In addition, resveratrol activated peroxisome proliferator-activated receptors, which play various roles in lipid and carbohydrate metabolism, agonist of PPARα. Resveratrol has been used as a hypolipidaemic drug reported to lower plasma and liver cholesterol levels in a PPARα-dependent manner (Tsukamoto *et al*., 2010).

Other benefits of resveratrol in obesity include its hypolipidaemic activity, increasing satiety, resting metabolic rate and a decrease in food intake, increased insulin sensitivity

(Miura *et al*., 2003; Lagouge *et al*., 2006; Nayagam *et al*., 2006; Sun *et al*., 2007; Dal-pan *et al*., 2010). Tumour necrosis factor-alpha (TNF-α) is chronically elevated in adipose tissues of obese rodents and humans. Results of studies suggest that resveratrol may improve obesity-induced cardiovascular disease, particularly atherosclerosis, by attenuating the TNF-alpha-induced changes of adipokines (Ahn *et al*., 2007).

2.17.13 *Resveratrol and Osteoporosis*

Resveratrol possesses oestrogenic activities, therefore it is known as a phytoestrogen. Resveratrol is a safe alternative to hormone replacement therapy in postmenopausal osteoporosis with reduced risk of breast cancer and cardiovascular disease. It has been shown to have protective effects, attributable to induction of bone morphogenetic protein-2 through Src kinase-dependent estrogen receptor activation (Chang *et al*., 2006; Su *et al*., 2007). Sehmisch *et al*. (2008) observed osteoprotective effects of resveratrol in ovariectomised rats, fed resveratrol for 12 weeks. In another study, resveratrol was investigated on blood pressure and bone loss in ovariectomized, stroke-prone spontaneously hypertensive rats. Resveratrol supplementation was observed to lower the systolic blood pressure by 15% and ovariectomy-induced decreases in femoral bone strength (Mizutani *et al*., 2000). Mizutani *et al*. (1998) also demonstrated that resveratrol stimulates cell proliferation and differentiation of osteoblasts. Similar studies in ovariectomized rats have also demonstrated that resveratrol exhibits osteoprotective properties (Lee *et al*., 2002; Li *et al*., 2005; Bottner *et al*., 2006).

2.18 Ethylenediaminetetraacetic Acid (EDTA)

Edetic acid (ethylenediaminetetraacetic acid) and its salts are commonly referred to as EDTA. Other names include N, N'-1, 2-ethanediylbis [N-(carboxymethyl) glycine], Versene acid, and (ethylenedinitrilo) tetraacetic acid. Molecular formula: $C_{10}H_{16}N_{2O}$

EDTA has been used extensively in medicine as a chelating agent for the removal of toxic heavy metals. The disodium salt of EDTA is a common component in many eye drops and contact lens wetting and cleansing solutions. EDTA is also used in a number of personal care and hygiene products, such as shampoos, liquid soaps, creams, and lotions. Household disinfectants often contain EDTA, especially if fatty acid soaps are used in the disinfectant formulation. These soaps are sensitive to calcium and magnesium, and the chelating agent prevents the formation of hard-water soap curds (Hart, 1984).

Experiments in humans also revealed poor absorption; only 2.5% of a 3-g dose of calcium disodium EDTA was excreted in the urine (Srbrova and Teisinger, 1957). Only 5% of a dose of 1.5 mg of 14 C-labelled calcium disodium EDTA given in a gelatin capsule to normal healthy men was absorbed (Foreman and Trujillo, 1954). EDTA has also been shown to be rapidly excreted from the body. Intravenous doses of 3 g of radiolabelled calcium disodium EDTA, given to two subjects, were almost entirely excreted within 12–16 hours (Srbrova and Teisinger, 1957).

A summary of a 1956 Ph.D. thesis by Chan in Anonymous (1964) reported biochemical studies with disodium EDTA. In a study in rats, 32 hours following administration of a single oral dose of 95 mg of disodium EDTA per rat, 93% of the dose was recovered from the colon. After doses of 47.5, 95, and 142.5 mg of disodium EDTA, the amount of

EDTA recovered in the urine was directly proportional to the dose given, suggesting that EDTA was absorbed from the gastrointestinal tract by passive diffusion.

2.18.1 *Acute Exposure with Ethylenediaminetetraacetic Acid*

Acute toxicity studies have been carried out with disodium EDTA and calcium disodium EDTA in laboratory animals. Median lethal dose (LD_{50}) values (mg/kg of body weight) reported in earlier studies are summarized below:

Rat

Oral:	2000–2200, Na2EDTA	(Yang, 1952)
Oral:	10 000 ± 740, CaNa2EDTA	(Oser *et al.*, 1963)

Rabbit

Oral:	2300, Na2EDTA	(Shibata, 1956)
Oral:	~7000, CaNa2EDTA	(Oser *et al*., 1963)
Intraperitoneal:	~500, CaNa2EDTA	(Bauer *et al*., 1952)

Dog

Oral:	~12 000, CaNa2EDTA	(Oser *et al.*, 1963)

Oser *et al*., (1963) reported that the oral LD_{50} in rats were not affected by the presence of food in the stomach or by pre-existing deficiency in calcium, iron, copper, or manganese

2.18.2 *Short-term exposure with Ethylenediaminetetraacetic Acid*

In a study involving the intraperitoneal administration of 250 or 500 mg of calcium disodium EDTA per kg of body weight per day to groups of five male rats for 3–21 days, it was reported that weight gain was satisfactory and that the histology of the lung, thymus, liver, spleen, adrenal, small gut, and heart was normal; there was a mild to moderate effect on the kidney (Reuber and Schmieller, 1962).

Groups of five male rats were given 250, 400, or 500 mg of disodium EDTA per kg of body weight per day intraperitoneally for 3–31 days; some groups were observed for an additional 2 weeks. At 500 mg/kg of body weight per day, all rats became lethargic and died within 9 days; the kidneys were pale and swollen, and there was moderate dilation of the bowel and subserosal haemorrhages. Histological examination of a number of organs showed lesions only in the kidneys. Animals at the 400 mg/kg of body weight per day dose level died within 14 days; kidney and bowel symptoms were similar to those seen at the high dose. One rat in the 250 mg/kg of body weight per day dose group showed haemorrhage of the thymus. All three groups exhibited varying degrees of damage to the kidney, with recovery occurring on withdrawal of the disodium EDTA (Reuber and Schmieller, 1962).

Four groups of one male and three female mongrel dogs were fed diets containing 0, 50, 100, or 200 mg of calcium disodium EDTA per kg of body weight per day for 12 months. At the end of the study, all dogs appeared to be well, and there were no significant changes in blood or urine analysis. Gross and microscopic examinations of the major organs were normal (Oser *et al*., 1963).

2.18.3 *Long-term exposure with Ethylenediaminetetraacetic Acid*

The long-term toxicity of EDTA is complicated by its ability to chelate essential and toxic metals, both in water and in animals. Toxicity data are therefore equivocal and difficult to interpret. An early study by Krum, (1948) demonstrated no adverse effect on weight gain, appetite, activity, and appearance in rats fed for 44–52 weeks on a diet containing 0.5% disodium EDTA.

A two-year study was carried out in which groups of rats were fed 0, 0.5, 1, or 5% disodium EDTA. The highest dose group showed a reduced food intake compared with the other groups and also suffered diarrhoea. No significant effects on weight gain were noted, nor were blood coagulation time, red blood cell counts, or bone ash adversely affected. Mortality of the animals could not be correlated with the level of disodium EDTA, as the highest mortality was observed in the control group and was due to pneumonia. Gross and microscopic examinations of the major organs did not reveal any significant differences between the groups (Yang, 1952).

In another study, groups of 25 male and 25 female rats were fed diets containing 0, 50, 125, or 250 mg of calcium disodium EDTA per kg of body weight per day for two years, and the study was carried on through four successive generations. The rats were mated after 12 weeks of feeding and were allowed to lactate for three weeks, with one week's rest before producing a second litter. Ten male and ten female rats from the F1 generation and similar F2 and F3 generations were allowed to produce two litters. With the second litter in the F1, F2, and F3 generations, only the control and the 250 mg/kg of body weight per day dose groups were 6 kept until the end of the two-year study on the F0 generation. No significant abnormalities in appearance or behaviour were noted during the 12 weeks of the post-weaning period in all generations. The experiments showed no statistically significant differences in weight gain, food efficiency, haematopoiesis, blood sugar, non-protein nitrogen, serum calcium, urine, organ weights, and histopathology of the liver, kidney, spleen, heart, adrenals, thyroid, and gonads (Oser *et al*., 1963).

Fifty weanling albino rats were fed a low-mineral diet (0.54% calcium and 0.013% iron) with the addition of 0, 0.5, or 1% disodium EDTA or0.5 or 1% calcium disodium EDTA

for 205 days. Diarrhoea was observed in the 1% disodium EDTA group, along with other abnormalities: growth retardation of the males, lowered erythrocyte and leukocyte counts, a prolonged blood coagulation time, slightly but significantly raised blood calcium level, a significantly lower ash content of the bone, and considerable erosion of the molars. Gross and histological examinations of the major organs revealed nothing abnormal. Rats fed for 220 days on an adequate mineral diet containing 1% disodium EDTA showed no evidence of dental erosion (Chan, 1956). Groups of 50 male and 50 female B6C3F1mice received trisodium EDTA in the diet at concentrations of 3, 750, or 7500 mg/kg of feed for 103 weeks, followed by 1 week during which the mice were fed standard diet without EDTA. The animals were examined twice per day for signs of toxicity. Gross and histopathological examinations of major organs and tissues were performed on animals found dead or moribund and on those sacrificed at the end of the study. Body-weight gain was decreased in high-dose males during the second year of the study, although no statistical analysis was presented. No treatment-related tumours or non-neoplastic lesions were observed in this study (NCI, 1977).

2.19 Succimer

Succimer is an analogue of dimercaprol (2, 3-dimercapto-1-propanol, British anti-lewisite, BAL), and has replaced dimercaprol as one of the main antidotes used in the management of poisoning by lead and other heavy metals. The advantages of succimer are that it is effective by oral administration because it is soluble in water; it is well-tolerated, has relatively low toxicity and can be given at the same time as iron supplements to treat iron deficiency anaemia. It does not cause significant increase in

urinary excretion of essential minerals unlike the other widely used lead chelating agent, sodium calcium EDTA (Bradberry and Vale, 2009).

Lead poisoning is a major public health concern around the world but many of the countries where the risks are greatest have only limited access to chelating agents. This application presents evidence to support addition of succimer to the WHO Essential Medicines List primarily for treatment of lead poisoning in children, for which it is most commonly used (Bradberry and Vale, 2009).

There is evidence that succimer is associated with short-term clinical improvement in lead poisoned children, but there is little evidence that asymptomatic children gain any long-term clinical benefit or improved long-term outcomes from reductions in blood lead concentrations (Bradberry and Vale, 2009).

Diagnosis of lead poisoning depends on blood lead concentrations, and physical examination focusing on neurological function. X-rays and haematological evaluation can support diagnosis but are not routine investigations.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 *Chemicals*

Trans-resveratrol (60g) of analytical grade was obtained from Candlewood Stars Incorporated, Danbury, USA (Batch Number: MR 110218), while lead acetate (product No; 10142, BDH Laboratory Chemicals Limited Poole, England) and carboxymethylcellulose- CMC (10 g) (Product No: 27929, BDH Laboratory chemicals limited Poole, England) were obtained from the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

3.1.2 *Equipment*

Some of the equipment's used in this study include Lead Care II User's Guide, Lead Care II blood analyser, Automated Haematology Analyzer (Sysmex model 2X-12N, USA). Automated Biochemistry Analyzer (Selectra XL, Vital Scientific, Netherlands) dissecting sets, syringes, and needles, spatula, regent bottles, digital weighing balance, sensors (2 containers of 24 each), treatment reagent tubes, capillaries/plungers, transfer droppers, calibration button, alcohol wipes, gauze pads, power free gloves were used.

3.1.3 *Experimental Animals*

Thirty six male wistar rats (150 - 250 g) were used in the study. The animals were housed in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were given access to pelletized growers marsh and water *ad libitum*. The rats were acclimatized for two weeks in the home cages and environment before commencement of the experiment. All experimental protocols were in accordance with the Ahmadu Bello University research policy (NIH publication number 85-23, revised 1996) and of

regulations governing the care and use of experimental animals. The experiments were conducted in a quiet environment between the hours of 0900 and 1600.

3.1.4 *Experimental Site*

The experiment was carried out between August- September, 2014 at the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria (11^{o} 10' N, 07^{o} 38' E), at the elevation of 650 m above sea level, located in the Northern Guinea Savannah zone of Nigeria (Akpa *et al*., 2002).

3.2 Methods

3.2.1 *Experimental Procedures*

3.2.1.1 *Resveratrol Preparation and Administration*

Trans-resveratrol, due to its low solubility in water, was suspended in 10 g/L Carboxymethylcellulose (CMC), and administered orally once daily for 14 days (Lia *et al.*, 2002; Juan *et al*., 2005).

3.2.1.2 *Lead Acetate Treatments and Resveratrol Pretreatment*

Male wistar rats were divided into six groups of six rats each. The first group served as negative control and recieved carboxymethylcellulose (CMC) 10 g/L body weight orally. The second, third, fourth and fifth groups recieved lead acetate at dose of 120 mg/kg (Magaji *et al.,* 2014b) body weight orally for 14 days while the sixth group was pretreated with resveratrol 400 mg/kg body weight (Magaji *et al.*, 2014a, Joanne *et al.*, 2008) orally for 5 days and serve as prophylaxis.

3.2.1.3 *Treatments with Succimer and Resveratrol*

After the lead acetate induction for 14 days and resveratrol pretreatment for 5 days, the treatment commenced on the 15^{th} day and lead acetate induction on the 5^{th} day, where

the second group positive control (lead poisoned), the third group was treated with succimer (10 mg/kg body weight) (POISINDEX, 2009; TOXBASE, 2009), the fourth group was treated with resveratrol (200 mg/kg body weight), the fifth group was treated with resveratrol (400 mg/kg body weight) orally for five (5) days and the sixth group was treated with lead acetate at dose of 120 mg/kg body weight orally for 14 days and served as prophylactic group (Magaji *et al.*, 2014a; Joanne *et al.*, 2008).

3.2.2 Acute Toxicity Test for Resveratrol

The limit test dose of 5000 mg/kg was used as stipulated in Organization of European Economic Community (OECD) guidelines (OECD, 2002). Four male wistar rats, each sequentially dosed at intervals of 48 h, were used for the test. The animals were observed individually for signs of toxicity and behavioural changes 1 h post-dosing, 24 h and at 48 h for 14 days.

3.2.3. Effect of Resveratrol on Relative Organ Weights in Lead-induced Toxicity in Wistar Rats

Animals were weighed daily using a Mettler weighing balance (Mettler Toledo Type BD6000, Greifensee, Switzerland). At the end of treatment duration (5 days), animals were fasted and euthanized on day 6 with chloroform. The essential organs including the liver, kidneys, spleen, heart, lungs, brain and testes were surgically harvested and weighed. Relative Organ Weight (ROW) was then calculated as follows:

$$ROW = \frac{AbsoluteOrganWeight(g)}{BodyWeightofratonsacrificeday(g)} \times 100$$

3.2.4 Induction of Lead Toxicity and Measurement of blood lead level (BLL)

In the six groups mentioned above (3.2.2.2 in p. 43), animals were assessed for clear signs of lead toxicity viz., weakness or aggressiveness, food refusal, loss of weight, diarrhea, discharge from eyes and ears, noisy breathing and mortality.

Measurements of blood lead level were carried out according to procedure provided by the Lead Care II Blood Lead Test kit manufacturers (Michigan Regional Laboratory System). The CDC laboratory first analyzed samples with ICP-MS (Jones *et al.,* 2009) using a modification of a method of Nixon *et al*., (1999) for analyses of metals in biological matrices. Fresh blood samples were analyzed on Lead Care II analyzer. Samples whose Lead Care II (LCII) results were greater than 65 μg/dL was reported as High Level (HI) on the Lead Care II and were prepared and analyzed using the blood dilution method. The goal of the dilution method was to dilute the sample to within the operating range of the Lead Care II (3.3 – 65 μg/dL) without changing the matrix of the blood and reagent-mixture against which the analyzer is calibrated. The method presented here required the diluent to be human or animal blood sample verified to have a BLL less than 3.3 μg/dL (reported on the analyzer as "Low level") using standard Lead Care II analytic methods on a calibrated machine using the test kit materials (ESA Biosciences Inc, 2007). This will be referred to as "low blood" in the subsequent steps. "Low blood" specimens obtained at each site were typically fresh (drawn within previous 24 hours), but were sometimes reused up to seven days, being stored at 2–8 °C when not in use. The "low blood" reagent-mixture to be used was prepared for analysis in an LCII reagent vial per the standard LCII protocol: 50 μL of the "low blood" specimen was transferred from the mixed (ideally with a vortex) tube of blood obtained from the carboxymethylcellulose

treated rats verified to have a BLL less than 3.3 μg/dL to an unused LCII reagent vial using an LCII capillary tube and plunger. This was followed by thorough mixing to create a "low blood" reagent-mixture with a total volume of 300 μL (250 μL of reagent and 50 μL of "low blood"). A "high blood" reagent-mixture was then prepared by using a capillary tube and plunger to transfer 50 μL of blood from the tube of the specimen previously determined by LCII analysis to have a BLL greater than 65μg/dL (reported as "HI" on the LCII) into a separate, unused, LCII reagent vial. Next, 50 μL of the "high blood" reagent-mixture was transferred to the vial containing 300 μL of "low blood" reagent-mixture using an unused LCII capillary tube and plunger, mixing well after the transfer. One drop of this final mixture was then analyzed on the LCII. Note that the LCII was calibrated to give results given a 1 + 5 dilution of blood upon adding 50 μL of blood to a 250 μL reagent container (50 μL in 300 μL of total solution = six fold dilution). By adding an already diluted 50–300 μL to create a total volume of 350 μL, there was an additional 1 + 6 dilution (50 μL in 350 μL of total solution = sevenfold dilution). Hence, to obtain the measured blood lead concentration in the "HI" sample, investigators simply multiplied the LCII result by 7.

3.2.5 Effect of Resveratrol on Haematological Parameters in Lead-induced Toxicity in Wistar Rats

Blood samples were collected at the end of treatment period after euthanasia on the 6th day. One portion (3 – 4 mls) was collected into K^+ EDTA bottles for estimation of packed cell volume (PCV), platelets, white blood cell count (WBC) and differentials using Automated Haematology Analyzer (Sysmex model 2X-12N, USA). The second portion is used for determination of biochemical parameters.

3.2.6 Effect of Resveratrol on Biochemical Parameters in Lead-induced Toxicity in Wistar Rats

Second portion (3 – 5 mls) of the blood sample from each animal after euthanasia was dispensed into plain bottles, allowed to clot and then centrifuged. The sera was separated and used for evaluation of biochemical parameters, which included alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) levels, total and conjugated bilirubin, serum urea and creatinine using Automated Biochemistry Analyzer (Selectra XL, Vital Scientific, Netherlands) and kits obtained from ELITech reagent kits, Netherlands.

3.2.7 Effect of Resveratrol on Histopathological Parameters in Lead-induced Toxicity in Wistar Rats

After the animals were euthanized, vital organs including liver, kidneys, spleen, heart, lungs, brain and testes were removed from the rats and fixed in 10% formalin for at least 48 h. But only liver, kidney, heart and brain were then processed routinely, and the tissues were embedded in paraffin wax. Histological sections were cut at 5 – 6 µm and stained with routine Periodic Acid Schiff (PAS) and haematoxylin and eosin (H & E) (Bancroft and Stevens, 1996). Detailed microscopic examinations were carried out by a consultant histopathologist. Photomicrographs of the organs were taken at various magnifications (× 100, × 250, and × 400)

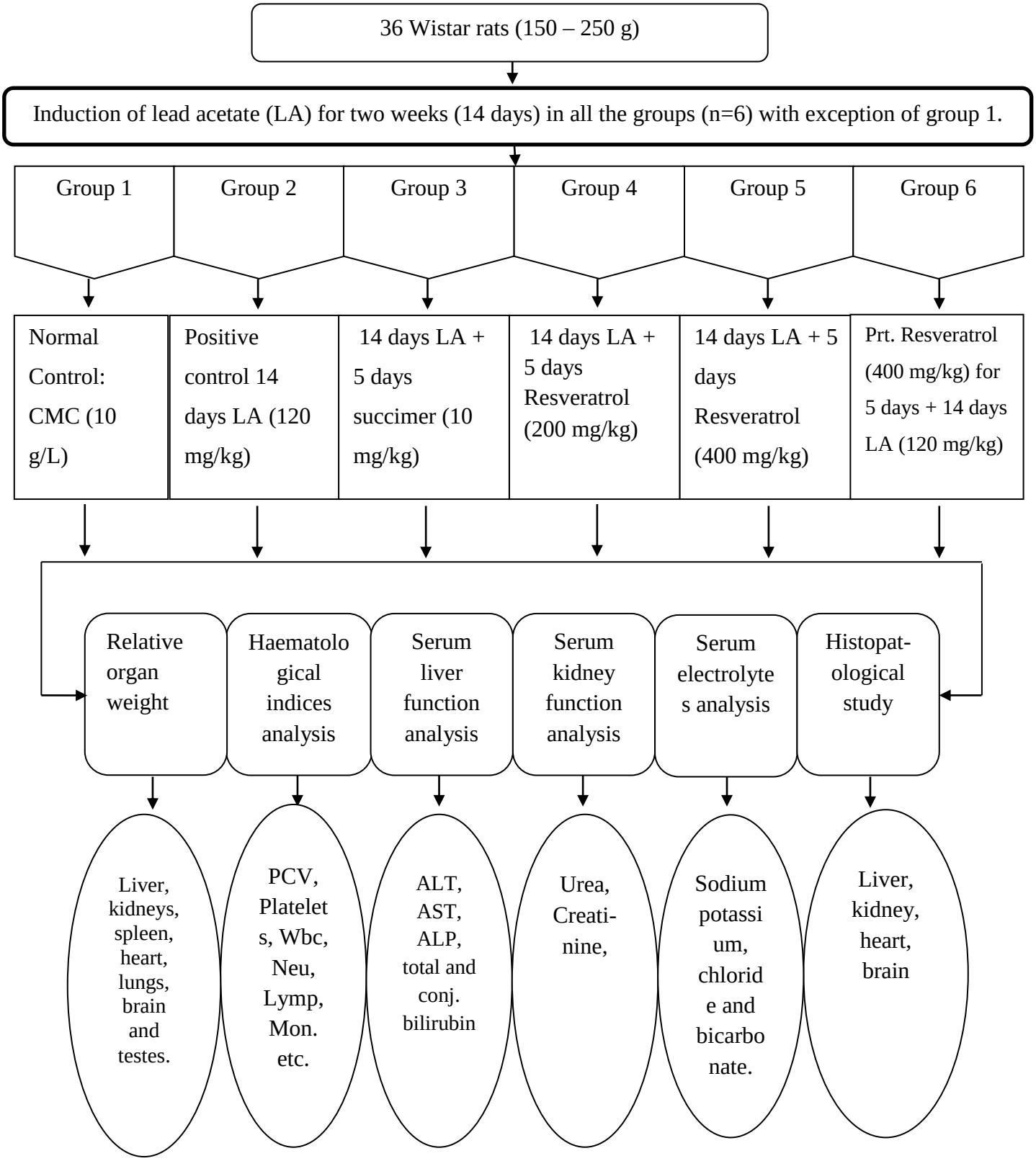


Fig. 3.1: Experimental Design for Sub-Acute Toxicity in wistar Rats (WHO, 1992 And OECD 407, 1995) Guidelines.

3.2.8 Statistical Analysis

Data obtained were expressed as mean ± SEM. Statistical analysis was carried out using SPSS version 20 and all the analysis were done using one way ANOVA followed by Tukey *post hoc* test for multiple comparisons. Values of $p < 0.05$ were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Acute Toxicity Study of Resveratrol

The limit dose of 5 g/kg did not cause mortality or any sign of acute toxicity in the four male wistar rats dosed for a short period (48 h) and long period (14 days).

4.2 Effect of Resveratrol on Body Weights of Lead-induced Toxicity in Wistar Rats

There was no statistically significant ($p > 0.05$) difference in body weights in resveratrol-treated groups when compared to both negative and positive control groups. But there was decrease in body weights in positive control group when compared to resveratrol-treated groups (Table 4.1)

Table 4.1 Effect of Resveratrol on Body Weights in Lead-induced Toxicity in Wistar Rats

TREATMENTS (mg/kg)	DAY 1	DAY 7	DAY 20
CMC (10 g/l)	191.00 ± 10.28	201.50 ± 11.37	209.50 ± 10.80
LA (120)	234.67 ± 07.41	227.83 ± 07.50	219.00 ± 07.72
LA (120) + S (10)	243.33 ± 03.78	216. 17 ± 11.22	234.33 ± 09.70
LA (120) + R (200)	216.00 ± 12.96	207.00 ± 13.10	230.50 ± 08.26
LA (120) + R (400)	165.83 ± 00.17	152.83 ± 01.45	185.00 ± 02.25
R (400) + LA (120)	152.50 ± 00.89	159.17 ± 01.42	158.83 ± 01.45

Values are presented as mean ± SEM (n=6). LA- Lead acetate, CMC-Carboxymethylcellulose, R-Resveratrol, S-Succimer

4.3 Effect of Resveratrol on Relative Organ Weights of Lead-induced Toxicity in Wistar Rats

There was no statistically significant ($p > 0.05$) difference in relative organ weights in resveratrol-treated groups when compared to both negative and positive control groups. But there was a decrease in relative organ weights in positive control group when compared to resveratrol-treated groups (Table 4.2)

Table 4.2 Effect of Resveratrol on Relative Organ Weights in Lead-induced Toxicity in Wistar Rats

Treatments (mg/kg)	Liver (%)	Kidney (%)	Heart (%)	Spleen (%)	Brain (%)	Testis (%)	Lung (%)
CMC (10 g/L)	3.85 ± 0.38	0.80 ± 0.09	0.38 ± 0.23	0.68 ± 0.07	0.89 ± 0.09	1.10 ± 0.16	2.42 ± 0.49
LA (120)	2.26 ± 0.13	0.56 ± 0.11	0.26 ± 0.01	0.31 ± 0.05	0.56 ± 0.03	0.95 ± 0.05	0.74 ± 0.07
LA (120) + S (10)	4.07 ± 1.11	0.91 ± 0.29	0.61 ± 0.27	0.60 ± 0.26	0.85 ± 0.23	1.51 ± 0.27	1.14 ± 0.38
LA (120) + R (200)	3.61 ± 0.08	0.78 ± 0.03	0.41 ± 0.02	0.51 ± 0.05	0.76 ± 0.03	1.30 ± 0.21	1.07 ± 0.04
LA (120) + R (400)	3.62 ± 0.13	0.71 ± 0.06	0.41 ± 0.05	0.55 ± 0.07	0.77 ± 0.06	2.03 ± 0.16	1.45 ± 0.18
R (400) + LA (120)	4.15 ± 0.13	0.96 ± 0.04	0.54 ± 0.03	0.59 ± 0.03	1.00 ± 0.05	2.01 ± 0.24	1.83 ± 0.22

Values are presented as mean ± SEM (n=6). LA- Lead acetate, CMC-Carboxymethylcellulose, R-Resveratrol, S-Succimer

4.4 Effect of Resveratrol on Blood Lead Level (BLLs) in Lead-induced Toxicity in Wistar Rats

There was a statistically significant ($p < 0.001$) decrease in the BLLs in Resveratrol-treated groups when compared to negative (carboxymethylcellulose 10 g/l) and positive (lead acetate 120 mg/kg) control groups (Fig.4.1)

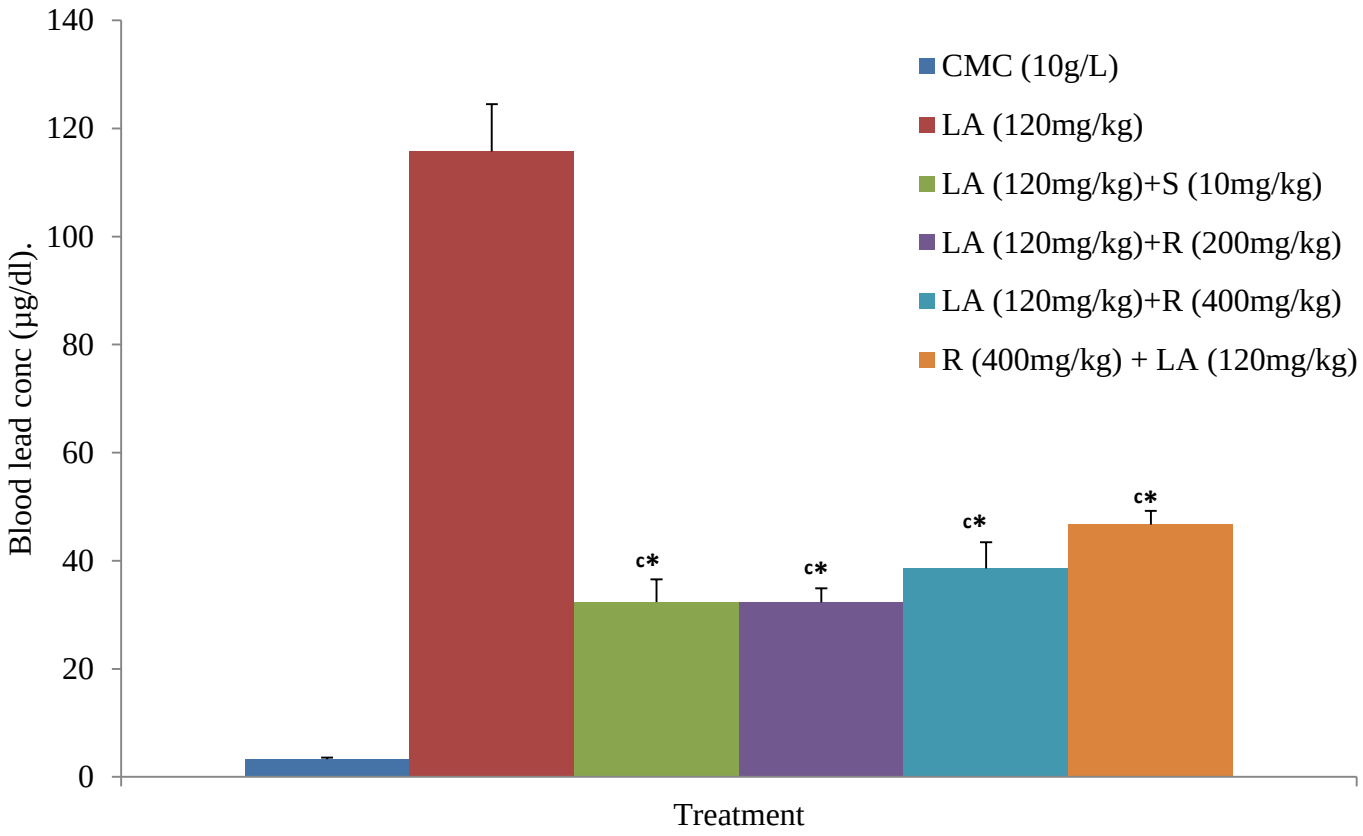


Fig. 4.1: Effect of Resveratrol on Blood Lead Levels in Lead-induced Toxicity in Male Wistar Rats. Values are presented as mean ± SEM. ^{c}* = $p < 0.001$ compared to CMC (10 g/l) and Lead acetate (120 mg/kg) one way ANOVA followed by tukey *post hoc* test. LA-Lead acetate, S-Succimer, R-Resveratrol, CMC-Carboxymthylcellulose

4.5 Effect of Resveratrol on Serum Kidney Function of Lead-induced Toxicity in Wistar Rats

There was no statistically significant ($p > 0.05$) difference in urea and creatinine levels in resveratrol-treated groups when compared to negative (carboxymethylcellulose 10 g/l) and positive (lead acetate 120 mg/kg) control groups (Table 4.3).

Table 4.3 Effect of Resveratrol on Serum kidney function in Lead-induced Toxicity in Wistar Rats

Treatments (mg/kg)	Urea levels (mmol/l)	Creatinine levels (mmol/l)
CMC (10 g/L)	6.88 ± 0.57	86.50 ± 8.39
LA (120)	9.10 ± 0.00	94.67 ± 3.67
LA (120) + S (10)	5.28 ± 1.00	80.00 ± 4.17
LA (120) + R (200)	4.48 ± 0.82	63.60 ± 5.96
LA (120) + R (400)	7.83 ± 1.85	86.67 ± 3.88
R (400) + LA (120)	6.83 ± 1.07	85.25 ± 5.53

Values are presented as means ± SEM (n=6). LA- Lead acetate, CMC-Carboxymethylcellulose, R-Resveratrol, S-Succimer

4.6 Effect of Resveratrol on Serum Electrolyte Levels of Lead-induced Toxicity in Wistar Rats

There is no statistically significant ($p > 0.05$) difference in sodium, potassium, chloride and bicarbonate levels (mmol/l) in resveratrol-treated groups when compared to negative (carboxymethylcellulose 10 g/l) and positive (lead acetate 120 mg/kg) control groups (Table 4.4)

Table 4.4: Effect of Resveratrol on Serum Electrolyte Levels in Lead-induced Toxicity in Wistar Rats

Treatments (mg/kg)	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	HCO_3^- (mmol/l)
CMC (10 g/L)	140.67 ± 02.79	4.52 ± 00.13	105.83 ± 03.51	23.17 ± 00.79
LA (120)	135.67 ± 07.84	17.67 ± 00.67	99.33 ± 09.68	21.67 ± 00.88
LA (120) + S (10)	135.40 ± 04.23	4.40 ± 00.43	96.40 ± 03.82	23.60 ± 00.51
LA (120) + R (200)	134.40 ± 06.21	4.26 ± 00.45	98.20 ± 07.25	23.00 ± 00.89
LA (120) + R (400)	130.50 ± 02.93	3.95 ± 00.28	93.50 ± 03.14	22.33 ± 00.84
R (400)+ LA (120)	142.00 ± 05.43	16.88 ± 12.04	102.75 ± 03.82	24.75 ± 01.49

Values are presented as means ± SEM (n = 6). LA- Lead acetate, CMC-Carboxymethylcellulose, R-Resveratrol, S-Succimer.

4.7 Effect of Resveratrol on Liver Function Parameters of Lead-induced Toxicity in Wistar Rats

There is no statistically significant ($p > 0.05$) difference in plasma levels of AST, ALT, ALP, total bilirubin and conjugated bilirubin in Resveratrol-treated groups when compared to negative and positive control groups. However the plasma levels of AST, ALT, ALP, total bilirubin and conjugated bilirubin were low in positive control group (lead acetate 120 mg/kg) in comparison with negative control (carboxymethylcellulose 10 g/l) and resveratrol-treated groups (Table 4.5).

Table 4.5 Effect of Resveratrol on Liver Function Parameters in Lead-induced Toxicity in Wistar Rats

Treatments (mg/kg)	AST levels (IU/L)	ALT levels (IU/L)	ALP levels (IU/L)	Total Bilirubin level (mmol/L)	Conjugated Bilirubin levels (mmol/L)
CMC (10 g/L)	33.00 ± 2.61	10.83 ± 02.20	206.17 ± 41.81	29.28 ± 06.95	9.37 ± 02.22
LA (120)	12.33 ± 2.73	05.33 ± 00.67	67.33 ± 29.81	24.00 ± 04.62	6.80 ± 01.20
LA (120) + S (10)	24.40 ± 5.48	12.00 ± 01.64	144.60 ± 28.83	52.28 ± 24.88	9.62 ± 03.70
LA (120) + R (200)	17.60 ± 2.77	15.20 ± 03.06	155.00 ± 15.72	44.68 ± 24.64	5.66 ± 00.96
LA (120) + R (400)	28.17 ± 3.39	12.00 ± 02.54	170.17 ± 21.45	92.08 ± 29.40	17.77 ± 04.92
R (400) + LA (120)	25.75 ± 8.22	15.50 ± 07.12	107.25 ± 27.83	40.98 ± 13.99	11.18 ± 03.01

Values are presented as means ± SEM (n = 6). LA- Lead acetate, CMC-Carboxymethylcellulose, R-Resveratrol, S-Succimer

4.8 Effect of Resveratrol on Haematological Parameters of Lead-induced Toxicity in Wistar Rats

There was a statistical significant ($p < 0.05$) increase in platelets count (392.33 ± 31.81 L/L) in Lead acetate (120 mg/kg) + Resveratrol (400 mg/kg) group when compared to positive control (Lead acetate 120 mg/kg) (210.50 ± 24.99 L/L) and negative control (carboxymethylcellulose 10 g/L) (219.50 ± 30.50 L/L) group. There was no statistical significant ($p > 0.05$) difference in PCV, WBC, neutrophils, lymphocytes and monocytes in resveratrol-treated groups when compared to negative (carboxymethylcellulose 10 g/l) and positive control groups (lead acetate 120 mg/kg) (Table 4.6).

4.6 Effect of Resveratrol on Haematological Parameters in Lead-induced Toxicity in Wistar Rats

Treatments (mg/kg)	Packed cell volumes (L/L)	White Blood Cells (L/L)	Platelets (L/L)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
CMC (10 g/L)	03.95 ± 03.53	32.83 ± 05.51	219.50 ± 30.50	25.17 ± 07.29	70.67 ± 06.71	04.60 ± 01.01
LA (120)	00.42 ± 00.03	14.33 ± 04.18	210.50 ± 24.99	22.83 ± 03.90	76.33 ± 03.98	03.00 ± 00.00
LA (120) + S (10)	00.39 ± 00.02	09.98 ± 03.30	261.00 ± 30.51	37.20 ± 04.96	59.60 ± 04.96	04.00 ± 01.56
LA (120) + R (200)	00.50 ± 00.02	12.16 ± 02.87	237.00 ± 19.72	15.80 ± 02.46	83.80 ± 02.25	_
LA (120) + R (400)	00.40 ± 00.02	19.18 ± 05.25	392.33 ± 31.81[a]*	38.00 ± 06.22	49.83 ± 05.93	03.50 ± 00.65
R (400) + LA (120)	00.44 ± 00.02	06.87 ± 00.41	181.67 ± 47.57	18.67 ± 02.91	81.33 ± 02.91	_

Values are represented as means ± SEM. [a]* = $p < 0.001$ compared to CMC (10 g/l) and Lead acetate (120 mg/kg) one way ANOVA followed by tukey *post hoc* test. LA-Lead-acetate, R- Resveratrol, CMC-Carboxymethylcellulose, S-Succimer.

4.9 Effect of Resveratrol on Liver Histopathology in Lead-induced Toxicity in Wistar Rats

Normal architecture of liver sinusoid with Kupffer cell; central vein, viable hepatocyte were observed in rats treated with carboxymethylcellulose (Plate 4.1 (1)). Rats that were treated with lead acetate showed an interrupted liver parenchyma with evidence of hyperemia in the liver sinusoids, complete congested central vein. There is damp focal necrosis of some hepatocytes and some appeared vacuolated (Plate 4.1 (2)). Rats that were treated with succimer after lead acetate induction for 2 weeks showed slightly distorted liver parenchyma evidenced by dilated sinusoids and prominent kuffer cells. Some hepatocytes appeared karyolytic and pyknotic conspicious (Plate 4.1 (3)). The rats treated with resveratrol (200 mg/kg) after lead acetate induction for 2 weeks showed an improved liver parenchyma. Liver hepatocytes look viable; however, there is some evidence of prominent kupffer cells and focal necrosis of some hepatocytes (Plate 4.1 (4)). Rats treated with resveratrol (400 mg/kg) after lead acetate induction for 2 weeks showed a preserved liver parenchyma, hepatocytes appear viable (Plate 4.1 (5)). Rats that were pretreated with resveratrol (400 mg/kg) for 5 days then lead acetate for 2 weeks showed preserved liver parenchyma, hepatocytes appear viable and prominent kupffer cells in sinusoids (Plate 4.1 (6)). Rats that were treated with distilled water for 2 weeks showed normal central vein and viable hepatocytes (Plate 4.1 (7)).

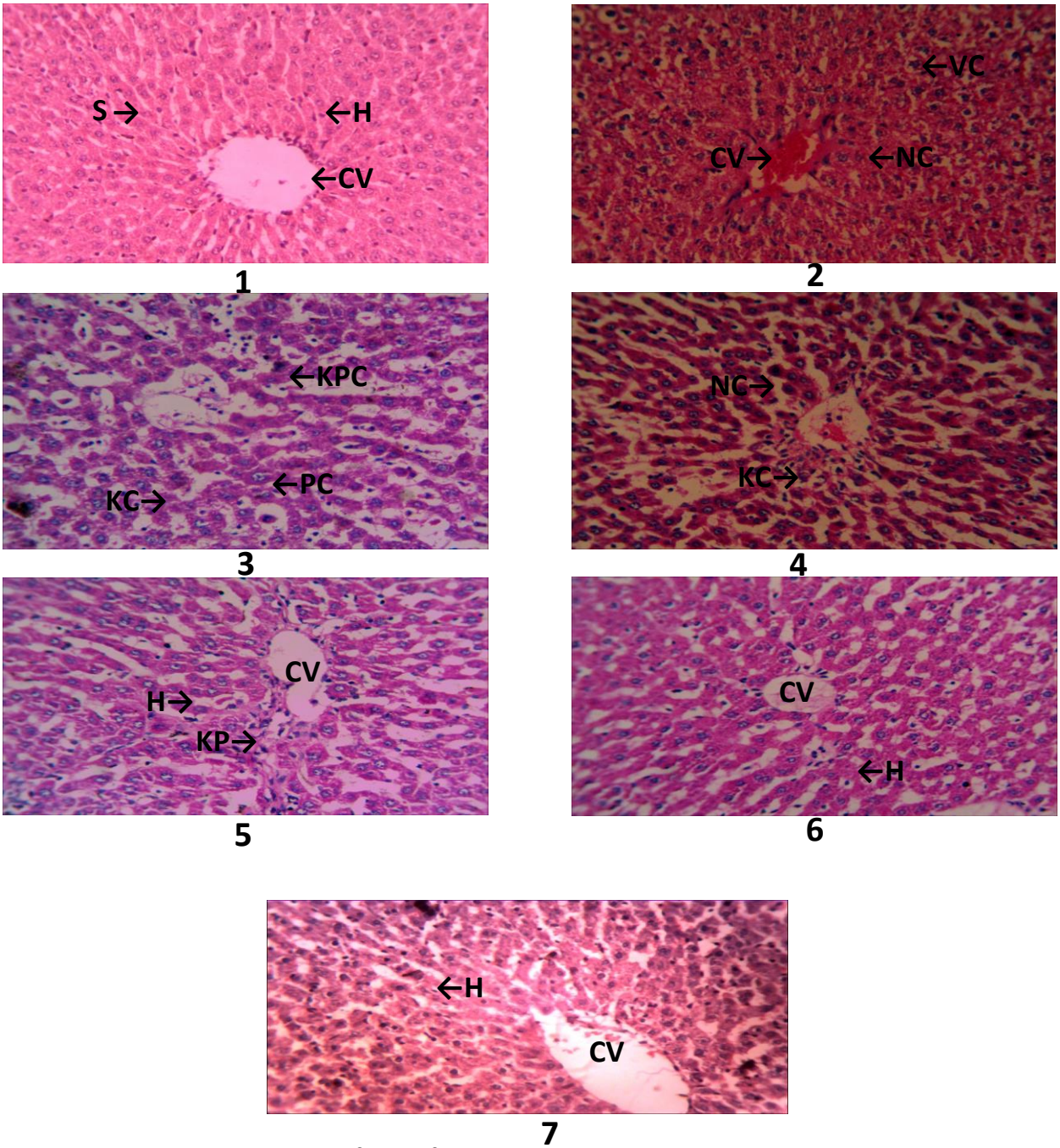


PLATE 4.1 Photomicrograph of liver from (1) Rats treated with carboxymethylcellulose (10g/L). Note liver sinusoid (S) with Kupffer cell; central vein (CV)-central vein; viable hepatocyte (H) (arrows), **(2)** Rats treated with lead acetate (120gm/kg). Note, congested central vein (CV); necrotic liver cell (NC) (hepatocyte); vacuolated hepatocyte (VC) (arrows), **(3)** Rats treated with succimer (10mg/kg). Note, Karyolytic cell (KC); pyknotic cell (PC); conspicious kupffer cells (KPC) (arrows), **(4)** Rats treated with resveratrol (200mg/kg). Note, necrotic cell (NC); conspicious kupffer cells (KC) (arrows), **(5)** Rats treated with resveratrol (400mg/kg). Note, viable hepatocyte (H); conspicious kupffer cells (KP) (arrows), central vein (CV), **(6)** Rats pretreated with resveratrol (400mg/kg) Note, viable hepatocyte (H) (arrows), central vein (CV). **(7)** Rats treated with distilled water. Note liver sinusoid (S) with Kupffer cell; central vein (CV); viable hepatocyte (H) (arrows), H&E x 250

4.10 Effect of Resveratrol on Liver Differential Hepatocyte Histopathology in Lead-induced Toxicity in Wistar Rats

The periodic acid schiff (PAS) stain result of the liver showed normal storage of glycogen deposit in hepatocytes (magenta colour) in carboxymethylcellulose treated rats (Plate 4.2 (1)). Lead acetate treated rat's liver showed a distortion in the pattern of glycogen storage in hepatocytes with diminished glycogen deposit (Plate 4.2 (2)). Succimer treated rat's liver showed slight improvement in the pattern of glycogen storage in hepatocytes with reduced glycogen deposit (Plate 4.2 (3)). Resveratrol (200 mg/kg) treated rat's liver showed a marked improvement in the pattern of glycogen storage in hepatocytes (Plate 4.2 (4)). Resveratrol (400 mg/kg) treated rats' liver showed a marked improvement in the pattern of glycogen storage in hepatocytes more than as seen in resveratrol (200 mg/kg) (Plate 4.2 (5)). Resveratrol pretreated rats' liver showed a marked improvement in the pattern of glycogen storage in hepatocytes more than as seen in resveratrol (400 mg/kg) treated rats (Plate 4.2 (6)). Distilled water treated rats liver showed normal storage of glycogen deposit in hepatocyte (Plate 4.2 (7)).

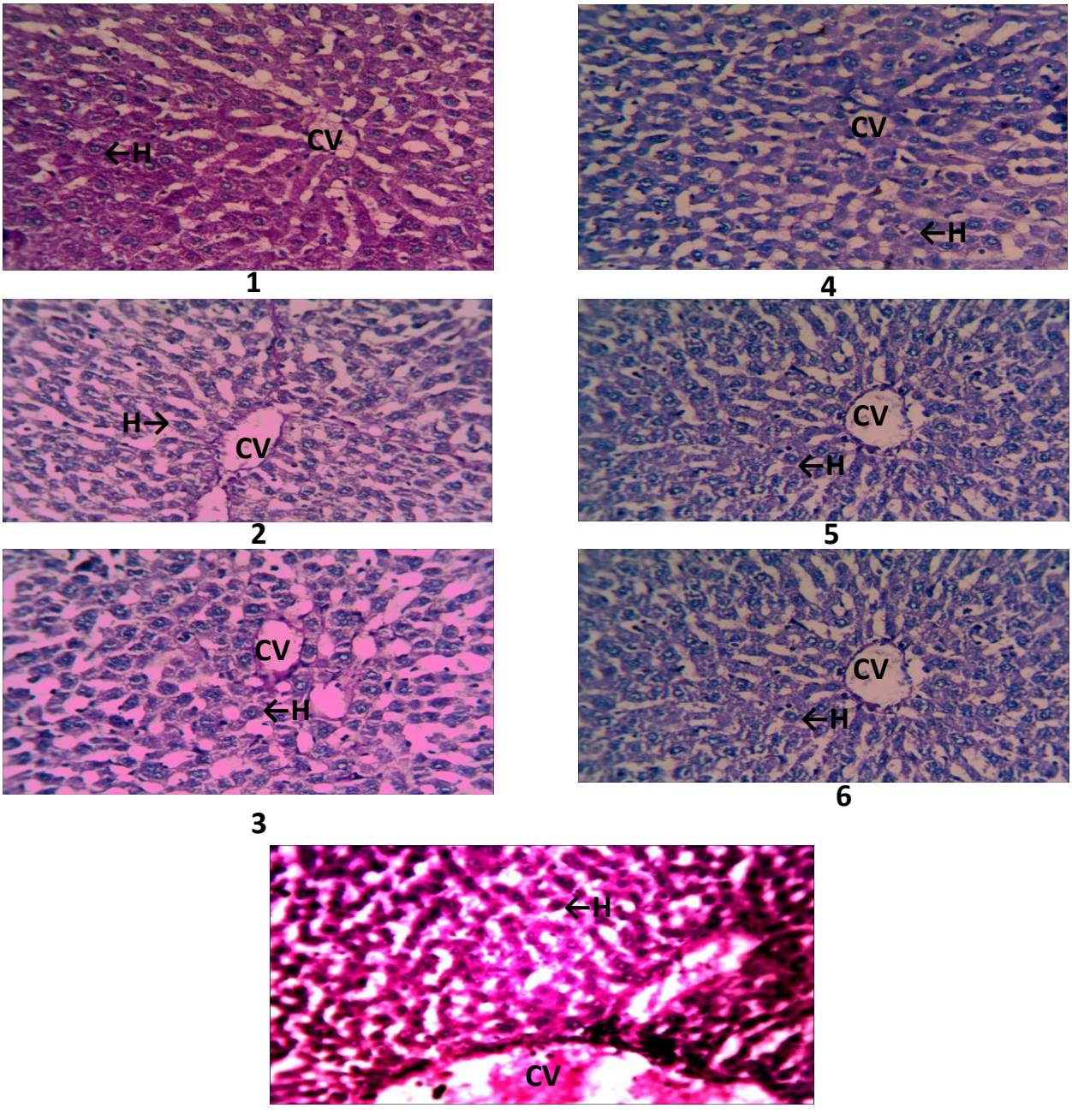


PLATE 4.2 Photomicrograph of liver from **(1)** Rats treated with carboxyl methylcellulose (10g/L). Note, hepatocyte (H) with glycogen deposit (magenta colour) (arrow); central vein (CV)**, (2)** Rats treated with lead acetate (120 mg/kg). Note –hepatocyte (H) with diminished glycogen deposit (arrow); CV-central vein**, (3**) rat treated with succimer (10mg/kg). Note – hepatocyte (H) with reduced glycogen deposit (arrow); CV-central vein, (**4)** Rats treated with resveratrol (200 mg/kg). Note –hepatocyte (H) (arrow); central vein (CV)**, (5)** Rats treated with resveratrol (400 mg/kg). Note –hepatocyte (H) (arrow); CV-central vein, (**6)** Rats treated with resveratrol (400 mg/kg) and lead acetate (120 mg/kg). Note –hepatocyte (H) (arrow); CV-central vein. **(7)** Rats treated with distilled water Note, hepatocyte (H) with glycogen deposit (magenta colour) (arrow); central vein (CV).PAS x 250.

4.11 Effect of Resveratrol on Cardiac Muscle Histopathology in Lead-induced Toxicity in Wistar Rats

Normal histology of the cardiac muscle was shown in negative control rats treated with carboxymethylcellulose with well-arranged cardiac muscle fiber and nucleus endothelial cells (Plate 4.3 (1)). Rats that were treated with lead acetate positive control showed a conspicious infiltration by macrophagic cells and loss in branching of muscle fibers (Plate 4.3 (2)). Rats that were treated with succimer after lead acetate induction for 2 weeks showed some loss in branching of the cardiac muscle fibers and to lesser degree than in positive control group. Little infiltration by some macrophagic cells was also observed (Plate 4.3 (3)). Rats that were treated with resveratrol (200 mg/kg) after induction of lead acetate for 2 weeks showed improved cardiac muscle architecture. However, there was slight evidence of macrophages (Plate 4.3 (4)). Rats that were treated with resveratrol (400 mg/kg) after induction of lead acetate for 2 weeks showed preserved cardiac muscle architecture. There is no significant histologic finding seen (Plate 4.3 (5)). Rats that were pretreated with resveratrol (400 mg/kg) for 5 days before 2 weeks lead acetate induction showed well preserved histology architecture as seen in resveratrol (400 mg/kg) treated (Plate 4.3 (6)). Distilled water treated rats showed normal cardiac muscle with well-arranged cardiac muscle fiber and endothelial cells (Plate 4.3 (7)).

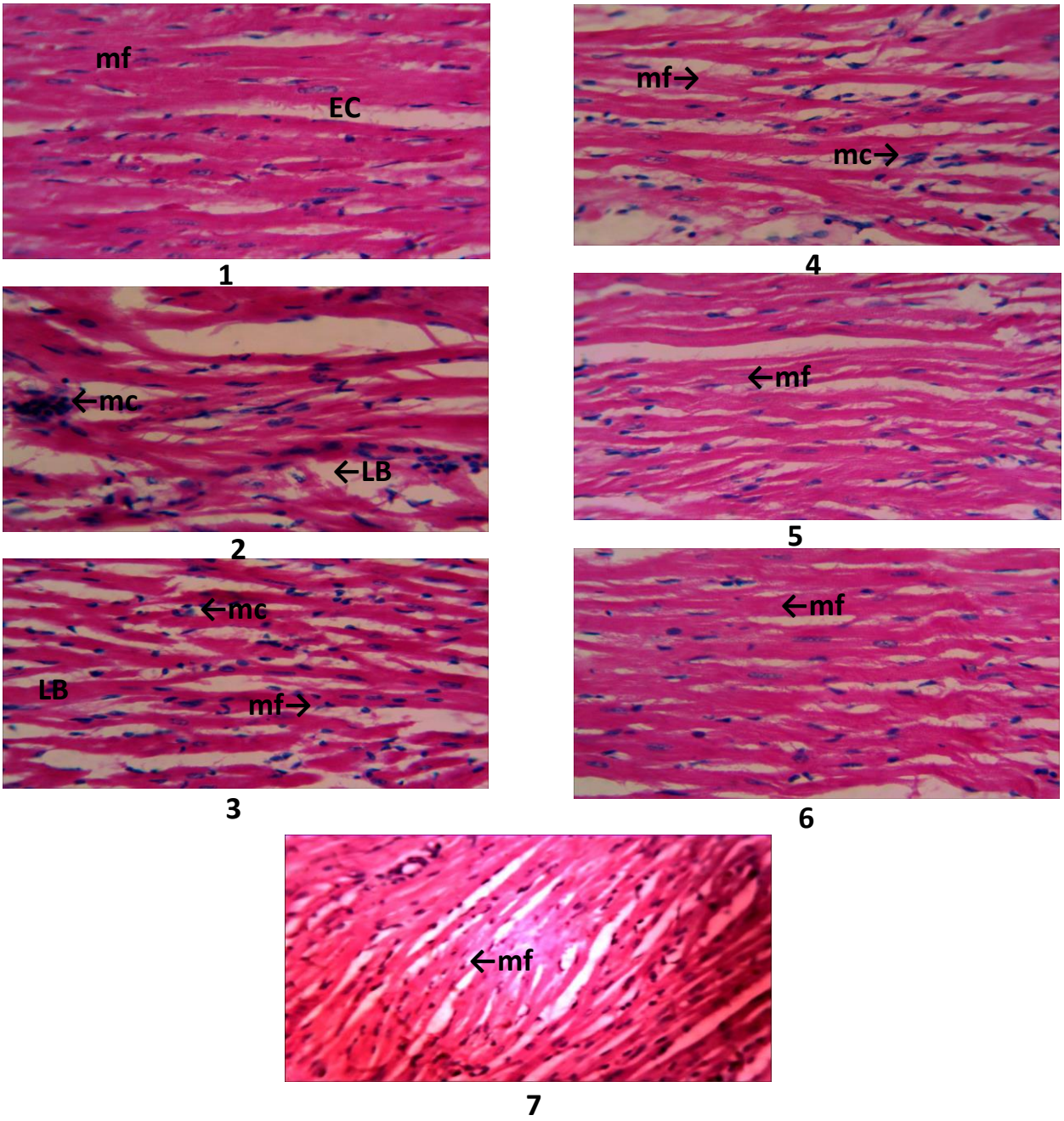


PLATE 4.3 Photomicrograph of cardiac muscle from (1) Rats treated with carboxyl methylcellulose (10g/L). Note, cardiac muscle fiber (MF) and nucleus; -endothelial cell (EC), **(2)** Rats treated with lead acetate (120mg/kg). Note, macrophagic cell (MC); area of loss in cardiac muscle branching (LB) (arrows), (**3)** Rats treated with succimer (10mg/kg). Note: cardiac muscle fiber (mf); -macrophagic cells (mc) (arrows); area of loss in branching (*lb),* (**4)** Rats treated with resveratrol (200mg/kg). Note, - cardiac muscle fiber (mf), macrophagic cell (mc) (arrows)**, (5)** Rats treated with resveratrol (400mg/kg). Note, cardiac muscle fiber (mf) (arrow)**, (6)** Rats treated with resveratrol (400mg/kg) and lead acetate (120mg/kg). Note, cardiac muscle fiber (mf) (arrow) **(7)** Rats treated with distilled water. Cardiac muscle fiber (mf), nucleus, endothelial cell (EC) H & E x 400.

4.12 Effect of Resveratrol on Kidney Histopathology in Lead-induced Toxicity in Wistar Rats

Kidney renal cortex of the carboxymethylcellulose treated rat's revealed normal histology of the renal cortex (Plate 4.4 (1)). Rats treated with lead acetate only showed a significant infiltration by macrophages (Plate 4.4 (2)). Rats treated with succimer showed an improved architecture of the renal cortex, however, there was slight infiltration by macrophages (Plate 4.4 (3)). Rats treated with resveratrol (200 mg/kg) showed improved renal cortex histology, thus, no significant histopathology seen (Plate 4.4 (4). Rats treated with resveratrol (400 mg/kg) showed well preserved renal cortex histology. Thus, no significant histopathology was seen (Plate 4.4 (5)). Rats pretreated with resveratrol (400 mg/kg) showed the same findings as that seen in resveratrol treated rats (Plate 4.4 (6)). Distilled water treated rats showed normal renal cortex of the kidney (Plate 4.4 (7)).

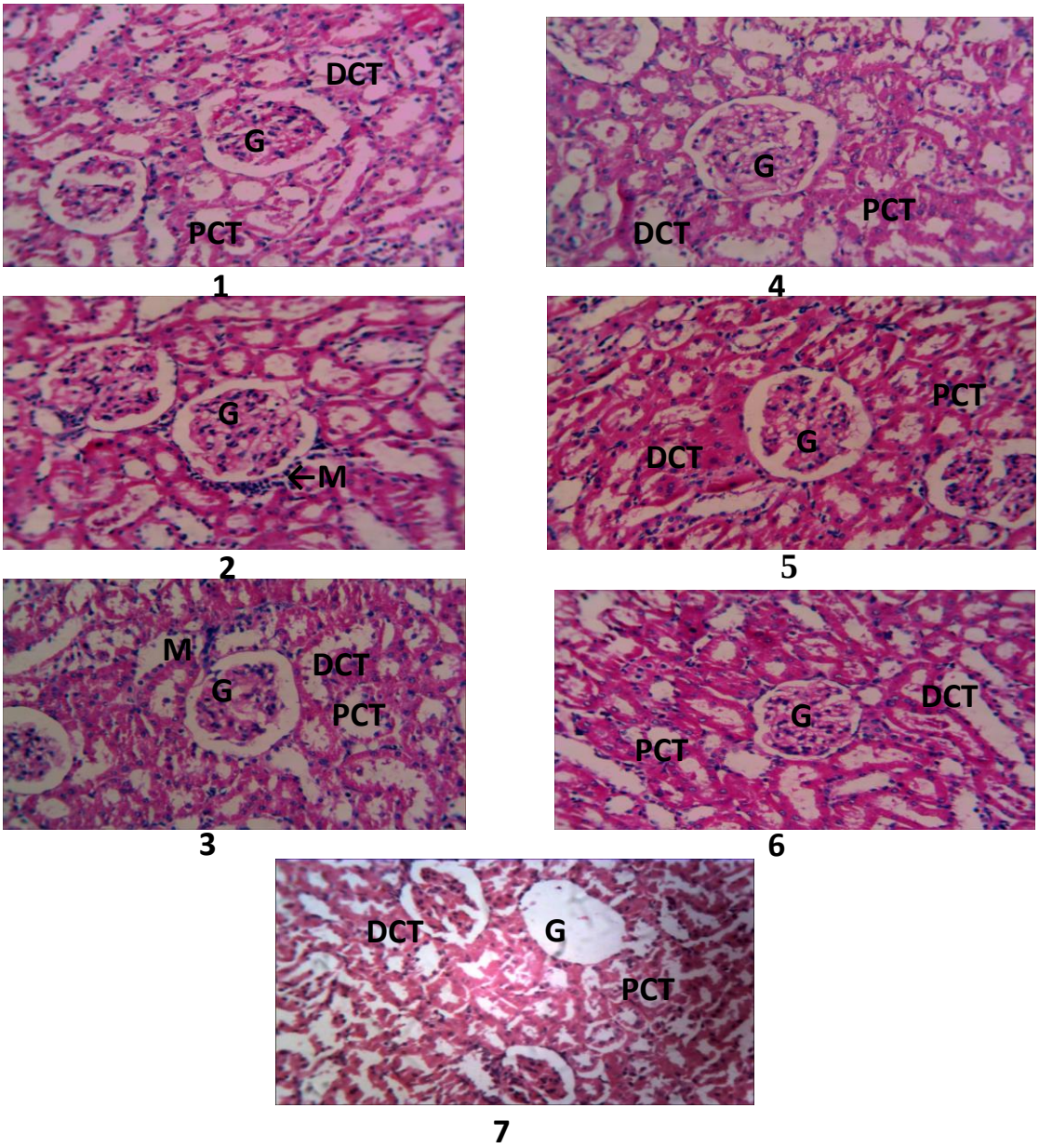


PLATE 4.4 Photomicrograph of kidney cortex from **(1)** Rats treated with carboxyl methylcellulose (10 g/L). Note, G-glomerulus (arrow); PCT-proximal convoluted tubule; DCT-distal convoluted tubule, (**2)** Rats treated with lead acetate (120 mg/kg). Note, G-glomerulus; M-macrophages (arrow), (**3)** Rats treated with succimer (10 mg/kg). Note, G-glomerulus; M-macrophages (arrow), **(4)** Rats treated with resveratrol (200 mg/kg). Note, G-glomerulus, PCT-proximal convoluted tubule, DCT-distal convoluted tubule, **(5)** Rats treated with resveratrol (400 mg/kg). Note, G-glomerulus, PCT-proximal convoluted tubule, DCT-distal convoluted tubule, (**6)** Rats treated with resveratrol (400 mg/kg) and lead acetate (120 mg/kg). Note, G-glomerulus, PCT-proximal convoluted tubule, DCT-distal convoluted tubule. **(7)** Rats treated with distilled water, Note, G-glomerulus (arrow); PCT-proximal convoluted tubule; DCT-distal convoluted tubule, H&E x 250

4.13 Effect of Resveratrol on Brain Histopathology in Lead-induced Toxicity in Wistar Rats

Normal architecture of the cerebral cortex of the brain was revealed in carboxymethylcellulose treated rats with molecular cell layer, pyramidal cell layer and granular cell layer well preserved (Plate 4.5 (1)). Rats treated with lead acetate only showed irregular contours of neurons, vacoulations in the cerebrum, degenerated neurons, and depletion of cells in granular layer of hippocampus with vacoulations. Thus, delamination of the cerebral cortex layers (Plate 4.5 (2)). Rats treated with succimer showed molecular cell layer, granular cell layer with slight delamination of pyramidal cell layer (Plate 4.5 (3)). Rats treated with resveratrol (200 mg/kg) showed well-arranged molecular cell layer, granular cell layer with slight distortion of delamination of pyramidal cell layer (Plate 4.5 (4)). Rats treated with resveratrol (400 mg/kg) showed more perfect arrangement in molecular cell layer, granular cell layer and pyramidal cell layer (Plate 4.5 (5)). Rats pretreated with resveratrol (400 mg/kg) showed similar findings as that seen in resveratrol (400 mg/kg) treated rats (Plate 4.5 (6)). Distilled water treated rats showed normal cerebral cortex of the brain with molecular, pyramidal and granular cell layers (Plate 4.5 (7)).

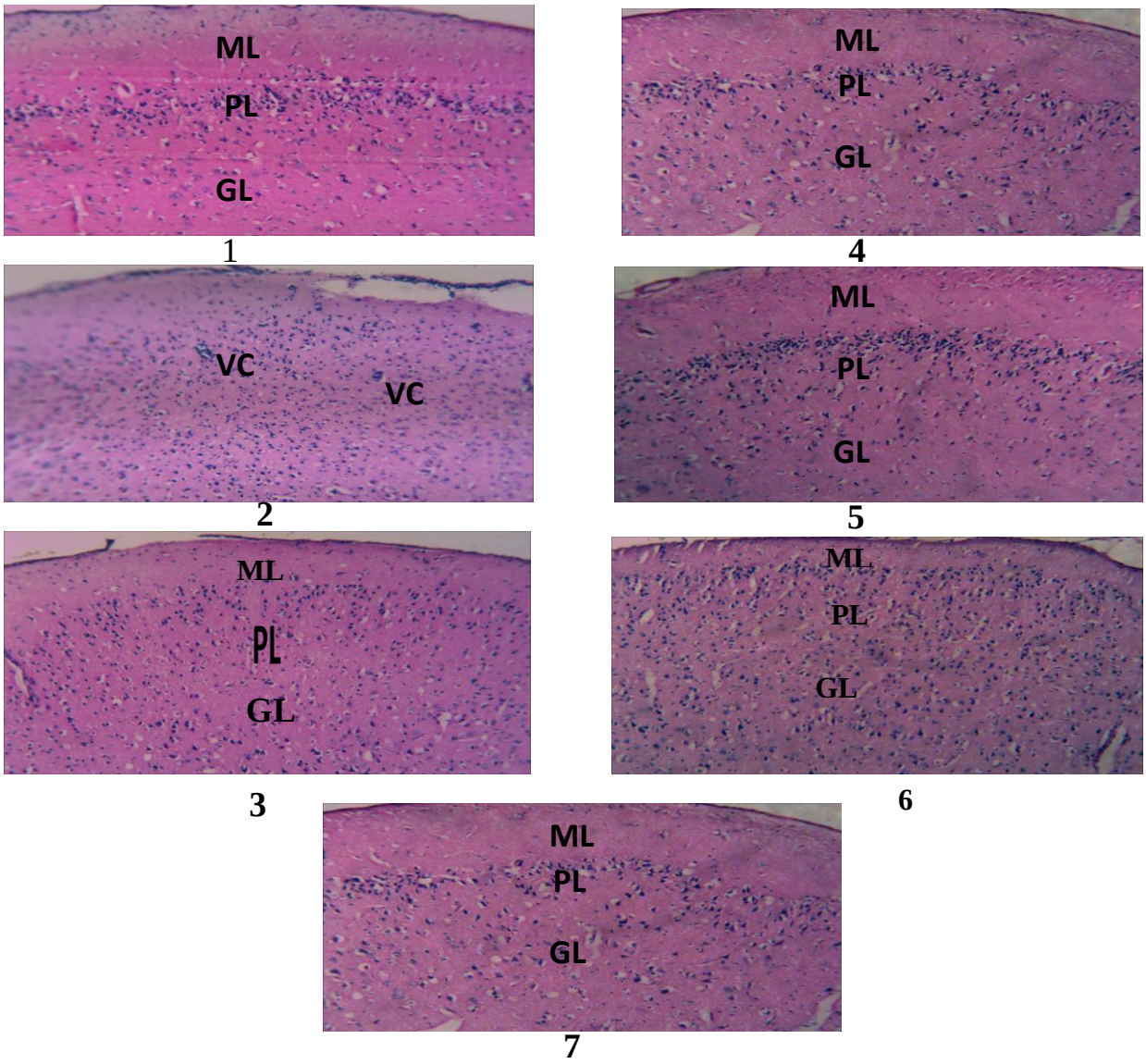


PLATE 4.5 Photomicrograph of cerebral cortex from **(1)** Rats treated with carboxyl methylcellulose (10g/L). Note, ML-molecular cell layer; PL-pyramidal cell layer; GL-granular cell layer, **(2)** Rats treated with lead acetate (120mg/kg). Note, irregular contours of neurons, vacoulations in the cerebrum (VC), degenerated neurons, depletion of cells in granular layer of hippocampus with vacoulations. NB: delamination of the cerebral cortex cell layers, (**3)** Rats treated with succimer (10mg/kg). Note, ML-molecular cell layer; PL-pyramidal cell layer; GL-granular cell layer. NB: slight delamination of the pyramidal cell layer**, (4)** Rats treated with resveratrol (200mg/kg).Note, ML-molecular cell layer; PL-pyramidal cell layer; GL-granular cell layer, (**5)** Rats treated with resveratrol (400mg/kg). Note, ML-molecular cell layer; PL-pyramidal cell layer; GL-granular cell layer, (**6)** Rats treated with resveratrol (400mg/kg) and lead acetate (120mg/kg). Note, ML-molecular cell layer; PL-pyramidal cell layer; GL-granular cell layer. **(7)** Rats treated with distilled water, Note, ML-molecular cell layer; PL-pyramidal cell layer; GL-granular cell layer, H & E x 100

CHAPTER FIVE

5.0 DISCUSSION

Lead is an ubiquitously found environmental and industrial pollutant that has been detected in nearly all phases of environment and biological system. Its persistence in human and animal tissues has quite often been associated with considerable health risks (Juberg *et al.,* 1997). This study was designed primarily to assess the possible ameliorative effects of resveratrol on lead induced organ toxicity in male wistar rats.

The results of the acute toxicity study indicated that the median lethal dose (LD_{50}) of the resveratrol was more than 5000 mg/kg. The limit test is primarily used in situations where the investigator has information indicating that the test material is likely to be non-toxic or of low toxicity (OECD, 2002). This finding, therefore, suggests that the resveratrol is essentially non-toxic and safe in oral formulation. This result is in line with previous studies (Walle *et al*., 2004; Crowell *et al.,* 2004; Brown *et al*., 2010), who reported LD_{50} of resveratrol in mice and rats to be more than 5000 mg/kg.

Organ weight can be the most sensitive indicator of an effect of an experimental compound, as significant differences in organ weight between treated and untreated (control) animals may occur in the absence of any morphological changes (Bailey *et al.,* 2004). The toxic signs observed in lead acetate treated rats in the present experiment were similar to the findings of other researchers such as Begum, (2004); Haque, (2005); Klauder and Petering, (1975). The loss in body and relative organ weights recorded in the lead acetate treated rats might have been as a result of loss of appetite and gastrointestinal disruption caused by the lead acetate.

Lowering of BLLs in resveratrol-treated groups may be due to antagonistic effect of resveratrol on lead. However, the exact nature of this antagonism which might include chelation would require further evaluation. Resveratrol pretreated rats also showed low BLLs compared to lead acetate treated rats which may be as a result of the pretreatment with resveratrol (400 mg/kg) before the lead acetate administration. Therefore, resveratrol may likely have protective effect against lead poisoning and this protection may appear to be dose related.

In the current study, the serum urea concentration was high in lead acetate treated wistar rats even though it wasn't significant. This suggested that the sub-acute administration of lead acetate evoked renal impairment since the kidney primarily eliminates urea in the urine (Ambali *et al.,* 2007) and it was associated with glomerular and renal tubular degeneration, partially evoked by oxidative stress. Similarly, El Neekety *et al.,*(2009) reported increased urea concentration in rats exposed to sub-acute lead acetate intoxication. It is postulated in this study that the increased urea concentration recorded in positive control group may be attributed to induction of renal damage. On the contrary, resveratrol treated and pretreated rats produced decreased urea concentration than lead acetate treated rats and this may be a demonstration of the nephroprotective role of resveratrol. This finding was in line with the findings of Roy *et al.,* (2009) and Das *et al.,* (2010).

Moreover, the elevated serum creatinine concentration in lead acetate treated rats may be an indication of renal damage and is in agreement with previous reports by Ghorbe *et al.,* (2001); Atef *et al.,* (1994) and Goel *et al.,* (2005). About 50% of kidney function must be lost before a rise in the serum concentration of creatinine can be detected (Kaptan and

Szabo, 1983). Therefore, urea, uric acid and creatinine could be considered as suitable prognostic indicators of renal dysfunction in case of lead exposure (Oberley *et al,* 1995 and Wang *et al*, 2002). However, glomerular function was still intact since the level of creatinine did not differ from the carboxymethylcellulose treated rats in resveratrol-treated groups.

Electrolytes are molecules that are electrically charged, which help move nutrients into and waste products out of the body's cells. They maintain healthy water balance and help stabilize the body's acid level. The data presented in this study suggests that alteration in sodium levels are caused by kidney disease and diuretics, and at times conditions that cause fluid to build up in the body. The most common cause of high sodium is dehydration (Halperin and Goldstein, 1994; Briggs *et al.,* 1996). The balance of sodium, chloride, potassium and bicarbonate ions in the body is a good indicator of how well the kidneys and heart are functioning. Chloride levels fluctuate with sodium levels. Low chloride levels can occur as a result of chronic lung disease, prolonged vomiting and metabolic alkalosis. High chloride levels can be due to kidney disease as well as dehydration. Change in serum chloride indicates an alteration in status and/or acid-base balance (Koch and Taylor, 1996). Acid-base balance is partly regulated by renal production and excretion of bicarbonate ions. Carbon dioxide in the form of bicarbonate is excreted and reabsorbed by the kidneys. High or low bicarbonate levels may signify acid/base or electrolyte imbalance often due to dehydration or drinking too much water. The primary regulators of bicarbonate are the proximal tubules (Halperin and Goldstein, 1994). From the data revealed, the reduction in serum sodium, bicarbonate and chloride

may be as result of tubular damage and necrosis as evidenced in the histopathological findings.

Analysis of the activities of basic liver function enzymes in serum are used to indirectly assess the integrity of tissues after exposure to pharmacological agents (Uboh *et al*., 2010). Serum enzyme measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue. ALP is an ectoenzyme of the plasma membrane whereas ALT and AST are enzymes of the cytosol (Shahjahan *et al.,* 2004). Increased serum levels of ALP, ALT and AST may suggest acute hepatotoxicity and are indicative of abnormalities in liver function (Obi *et al.*, 2004). Release of AST and ALT is as a result of injury to the liver (Giboney, 2005) while elevated level of ALP could be as a result of injury to the liver, bone, leucocytes, kidneys, intestines or placenta. The lack of significant alterations in the levels of ALT, AST, ALP, total and conjugated Bilirubin levels, which are good indicators of liver functions, suggests that sub-acute administration of resveratrol neither altered hepatocytes of rats nor the normal metabolism of the animals as evidence in histopathology findings. The observed low levels of serum liver function parameters in lead acetate treated rats is unusual because it is expected that the serum ALT, AST and ALP should have raised due to damage caused by lead acetate as evidenced in the liver histopathological findings where the liver showed necrosis of the liver cell, congested central vein and vacuolated hepatocytes. Moreover, this finding was totally in disagreement with the findings of Gill *et al.,* (1991); Moussa and Bashandy, (2008) who recorded elevated ALP, AST and ALT activity, which was used as a marker of liver adaptation to damaging factor in lead-exposed animals. Although this may be as a result

of autolysis of the enzymes due to dose concentration of lead acetate, however, the exact mechanism of enzyme degradation will require further evaluation.

Blood platelet aggregation under physiological conditions is an important process that arrests bleeding, but excessive platelet aggregation causes thrombosis and atherosclerosis (Olas and Wachowicz, 2005; Malinowska and Olas, 2011). The haemopoietic system serves as important target for toxic chemicals and is a sensitive index of pathological conditions. In the present study, treatment with resveratrol did not cause any alteration in haematological parameters except in the platelet (PLT) level. Increase in platelets count observed in resveratrol (400 mg/kg) treated rats might be an indication of resveratrol non-toxic effects on the bone marrow and it is possible that prolonged consumption of resveratrol could prevent thrombocytopenia (reduced PLT level), preventing bleeding disorders, due to its ability to cause an increased platelet count at dose of 400 mg/kg. Although, this finding was actually in disagreement with the finding of Malinowska and Olas, (2011) who demonstrated that resveratrol significantly reduced platelet count but in hyperhomocysteinaemia induced wistar rats as against the present study which used lead-induced toxicity in male wistar rats. Thus, the effect of resveratrol in PLT level is dose dependent. This study also observed that all treated rats had normal levels of packed cell volume (absence of anemia). However, anemia has been reported following lead poisoning which was attributed to various inhibitory effects of lead on heme biosynthesis (Kim *et al.*, 2002). Besides, excessive lead exposure inhibits the body's ability to make hemoglobin by interfering with several enzymatic steps in the heme pathway, through inhibiting aminolevulinic acid dehydratase and ferrochelatase activity, leading to anemia and erythrocytes degeneration or destruction (Patocka and Cerný, 2003).

In toxicological studies, histopathological examination provides supportive evidence for biochemical and haematological observations (Eroschencho, 2000). In the present study, the levels of Pb in tissues of liver were significantly higher in positive control group which results in necrosis of liver cell (hepatocyte) and vacuolated hepatocyte. Disruption of pro-oxidant/antioxidant balance might result in the tissue injury. In addition to an interrupted liver parenchyma with evidence of hyperemia in the liver sinusoids, complete congested central vein, there was damp focal necrosis of some hepatocytes and some appear vacuolated. Ingestion of Pb is one of the primary causes of its hepatotoxic effects. The molecular understanding of Pb effects on hepatic drug metabolizing enzymes, cholesterol metabolism, oxidative stress, and hepatic hyperplasia suggest a potential role for Pb in damaging extrahepatic systems, including the cardiovascular system. While treatment with succimer showed slightly distorted liver parenchyma evidenced by dilated sinusoids and prominent kupffer cells, some hepatocytes appear karyolytic and pyknotic conspicious. Furthermore, resveratrol (200 mg/kg and 400 mg/kg) showed an improved and preserved liver parenchyma. Liver hepatocytes looked viable; however, there was evidence of prominent kupffer cells and focal necrosis of some hepatocytes. Also, similar results were reported by Shalan *et al.,* (2005), Badiei *et al.,* (2006), Khan *et al.,* (2008). A similar result was also seen in group pretreated with resveratrol (400 mg/kg) before lead acetate induction. Similar result was reported by Ebuehi *et al.,* (2012) who used Vitamin E as supplement. However, these preserved and prominent liver hepatocytes which look viable and kupffer cells observed in resveratrol treated and pretreated animals is suggested to be as a result of chelating property of resveratrol. Patra *et al*., (2001) found a significant higher levels of lead in liver of rats exposed to Pb for 4 weeks and a

significant reduction of Pb levels after treatment with chelating agent, EDTA, after 5 week of treatment (Gary and Blair, 2007; Shalan *et al*., 2005). The most common and constant findings was a portal leukocytic infiltration, hydropic degeneration and loss of normal architecture in the liver. Resveratrol reduced tissue lead burden; the oral administration of resveratrol to lead-intoxicated rats augmented the antioxidant potential by affecting the antioxidant enzyme activities besides reducing the tissue injury of liver cells (Sahin *et al*., 2012).

The periodic acid schiff (PAS) stain result of the liver revealed normal storage of glycogen deposit in hepatocytes (magenta colour) in carboxymethylcellulose treated rats. Lead acetate treated rats liver revealed a distortion in the pattern of glycogen storage in hepatocytes with diminished glycogen deposit which may be as a result of the effect of the lead acetate administered. The administration of succimer and resveratrol revealed improvement in the pattern of glycogen storage in succimer, resveratrol treated and resveratrol pretreated rats. This may be as a result of reduced intoxication of lead acetate in those groups or may be as a result of chelating properties of succimer and resveratrol.

Examination of the kidney of animals treated with carboxymethylcellulose showed normal histological architecture of kidney cortex with clear glomerulus, proximal convoluted tubule, and distal convoluted tubule. Lead acetate treated animals revealed focal replacement of renal parenchyma by lymphocytes, macrophages and coagulative necrosis. Similar histopathological lesions have been reported in experimental lead acetate toxicity with different species by Durgut *et al.,* (2008), Rader *et al.,* (1983) and Vyskocil *et al.,* (1995). Lead acetate treated animals also revealed intranuclear inclusion

bodies in kidney proximal tubules. Some scholars also reported similar changes when they exposed animals to lead (Mohamed and Saleh, 2010). Furthermore, resveratrol treated and animals happened to have corrected most of the destructions made by lead acetate by improving the architecture of the kidney looking almost similar to that of the negative control group. This study also revealed that, the effectiveness of resveratrol is dose dependent because rats treated and pretreated with resveratrol 400 mg/kg showed more improvement by restoring the normal architecture of the kidney as seen in the negative control group. Similar findings were reported by Ashour *et al.,* (2007) and Ishiaq *et al.,* (2011).

Normal histology of the cardiac muscle showed in carboxymethylcellulose treated rats with well-arranged cardiac muscle fiber and nucleus endothelial cells. Lead acetate treated animals revealed a conspicious infiltration by macrophagic cells and loss in branching of muscle fibers, which was as a result of toxicities caused by the lead acetate administered. Rats that were treated with succimer after lead acetate induction for 2 weeks showed some loss in branching of the cardiac muscle fibers and to lesser degree than in lead acetate treated animals. Little infiltration by some macrophagic cells was also observed. Rats that were treated with resveratrol (200 mg/kg) after induction of lead acetate for 2 weeks showed improved cardiac muscle architecture. However, there is slight evidence of macrophages but there was restoration of the loss of branching of muscle fibers to some degree. Higher dose of with resveratrol (400 mg/kg) (group 5) after induction of lead acetate for 2 weeks showed preserved cardiac muscle architecture and was dose dependent. Rats that were pretreated with resveratrol (400 mg/kg) for 5 days followed 2 weeks lead acetate induction showed well preserved histology architecture.

This is an indication that resveratrol at dose of 400 mg/kg may have protective effect against lead poisoning.

The brain is an essential organ of the body which is reported to be impaired on exposure to lead with respect to time. A number of researches have reported impairment in brain architecture and function on exposure to lead (Ha *et al*., 2010, Xu *et al.*, 2009, Cecil *et al*., 2008). Lead acetate treated animals showed irregular contours of neurons, vacoulations in the cerebrum, degenerated neurons, and depletion of cells in granular layer of hippocampus with vacoulations, thus, delamination of cerebral cortex layers resulted due to the effect of lead intoxication. The present findings were in agreement with the findings of Xu *et al.,* (2009) and Ha *et al*., (2010). In the brain, Succimer and resveratrol treated animals showed some degree of improvement as it revealed normal molecular, pyramidal and granular cells layers of hippocampus with absence of vacoulations and delamination of cerebral cortex layers. Hassan and Jassim, (2010) reported similar findings with protective effects of some chemical agents (Vitamin C and E) against lead induced brain damage.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

In this study, it was observed that resveratrol increased body and relative organ weights in lead-induced toxicity in male wistar rats and significantly decreased BLLs. Resveratrol also significantly increased platelet counts (392.33 ± 31.8) in lead-induced toxicity in male wistar rats. Resveratrol's effectiveness in lead-induced toxicity in male wistar rats was dose dependent. In other words, it significantly ameliorated the adverse effect of lead-induced toxicity in male wistar rats.

6.2 Conclusion

In conclusion, resveratrol had ameliorated the adverse effects of lead-induced organ toxicities in male wistar rats.

6.3 Recommendations

Based on the findings of this study, the following is recommended:

i. Encourage the use of natural food stuff and fruits rich in resveratrol as a supplement in management of lead poisoning.

ii. Further studies should be carried out using longer period of experimental treatment with resveratrol and chronic exposure of lead acetate with variation in dosage. Also, for prophylactic studies, in addition to pre lead treatment with resveratrol, resveratrol treatment should continue concurrently with lead treatment.

iii. Other studies should be carried out in human subjects to assess the effectiveness of resveratrol in humans, affected with lead poisoning.

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APPENDIX I

Table 4.1 Effect of Resveratrol on Body Weights in Lead-induced Toxicity in Wistar Rats.

TREATMENTS (mg/kg)	DAY 1	DAY 7	DAY 20
CMC (10 g/l)	191.00 ± 10.28	201.50 ± 11.37	209.50 ± 10.80
LA (120)	234.67 ± 7.41	227.83 ± 7.50	219.00 ± 7.72
LA (120) + S (10)	243.33 ± 3.78	216. 17 ± 11.22	234.33 ± 9.70
LA (120) + R (200)	216.00 ± 12.96	207.00 ± 13.10	230.50 ± 8.26
LA (120) + R (400)	165.83 ± 0.17	152.83 ± 1.45	185.00 ± 2.25
9R (400) + LA (120)	152.50 ± 0.89	159.17 ± 1.42	158.83 ± 1.45

Values are presented as means ± SEM (n=6). LA- Lead acetate, CMC-Carboxymethylcellulose, R-Resveratrol, S-Succimer

APPENDIX II

Table 4.2 Effect of Resveratrol on Relative Organ Weights on Lead-induced Toxicity in Wistar Rats.

Treatments	Liver (%)	Kidney (%)	Heart (%)	Spleen (%)	Brain (%)	Testis (%)	Lung (%)
CMC (10 g/L)	3.85 ± 0.38	0.80 ± 0.09	0.38 ± 0.23	0.68 ± 0.07	0.89 ± 0.09	1.10 ± 0.16	2.42 ± 0.49
LA (120 mg/kg)	2.26 ± 0.13	0.56 ± 0.11	0.26 ± 0.01	0.31 ± 0.05	0.56 ± 0.03	0.95 ± 0.05	0.74 ± 0.07
LA (120 mg/kg) + S (10 mg/kg)	4.07 ± 1.11	0.91 ± 0.29	0.61 ± 0.27	0.60 ± 0.26	0.85 ± 0.23	1.51 ± 0.27	1.14 ± 0.38
LA (120 mg/kg) + R (200 mg/kg)	3.61 ± 0.08	0.78 ± 0.03	0.41 ± 0.02	0.51 ± 0.05	0.76 ± 0.03	1.30 ± 0.21	1.07 ± 0.04
LA (120 mg/kg) + R (400 mg/kg)	3.62 ± 0.13	0.71 ± 0.06	0.41 ± 0.05	0.55 ± 0.07	0.77 ± 0.06	2.03 ± 0.16	1.45 ± 0.18
R (400 mg/kg) + LA (120 mg/kg)	4.15 ± 0.13	0.96 ± 0.04	0.54 ± 0.03	0.59 ± 0.03	1.00 ± 0.05	2.01 ± 0.24	1.83 ± 0.22

Values are represented as mean ± SEM. CMC- carboxymethylcellulose, LA- Lead acetate, R- Resveratrol, S- Succimer

APPENDIX III

Table 4.3 Effect of Resveratrol on Electrolytes in Lead-induced Toxicity in Wistar Rats.

Treatments	Urea levels (mmol/l)	Sodium levels (mmol/l)	Potassium levels (mmol/l)	Chloride levels (mmol/)	Bicarbonate levels (mmol/l)	Creatinine levels (mmol/l)
CMC (10 g/L)	6.88 ± 0.57	140.67 ± 2.79	4.52 ± 0.13	105.83 ± 3.51	23.17 ± 0.79	86.50 ± 8.39
LA (120 mg/kg)	9.10 ± 0.00	135.67 ± 7.84	17.67 ± 0.67	99.33 ± 9.68	21.67 ± 0.88	94.67 ± 3.67
LA (120 mg/kg) + S (10 mg/kg)	5.28 ± 1.00	135.40 ± 4.23	4.40 ± 0.43	96.40 ± 3.82	23.60 ± 0.51	80.00 ± 4.17
LA (120 mg/kg) + R (200 mg/kg)	4.48 ± 0.82	134.40 ± 6.21	4.26 ± 0.45	98.20 ± 7.25	23.00 ± 0.89	63.60 ± 5.96
LA (120 mg/kg) + R (400 mg/kg)	7.83 ± 1.85	130.50 ± 2.93	3.95 ± 0.28	93.50 ± 3.14	22.33 ± 0.84	86.67 ± 3.88
R (400 mg/kg) LA (120 mg/kg)	6.83 ± 1.07	142.00 ± 5.43	16.88 ± 12.04	102.75 ± 3.82	24.75 ± 1.49	85.25 ± 5.53

Values are presented as mean ± SEM. CMC- carboxymethylcellulose, LA- Lead acetate, R- Resveratrol, S- Succimer.

APPENDIX IV

Table 4.4 Effect of resveratrol on Liver Functions in Lead-induced Toxicity in Wistar Rats.

Treatments	AST levels (IU/L)	ALT levels (IU/L)	ALP levels (IU/L)	Total Bilirubin level (mmol/L)	Conjugated Bilirubin levels (mmol/L)
CMC (10 g/L)	33.00 ± 2.61	10.83 ± 2.20	206.17 ± 41.81	29.28 ± 6.95	9.37 ± 2.22
LA (120 mg/kg)	12.33 ± 2.73	5.33 ± 0.67	67.33 ± 29.81	24.00 ± 4.62	6.80 ± 1.20
LA (120 mg/kg) + S (10 mg/kg)	24.40 ± 5.48	12.00 ± 1.64	144.60 ± 28.83	52.28 ± 24.88	9.62 ± 3.70
LA (120 mg/kg) + R(200 mg/kg)	17.60 ± 2.77	15.20 ± 3.06	155.00 ± 15.72	44.68 ± 24.64	5.66 ± 0.96
LA (120 mg/kg) + R (400 mg/kg)	28.17 ± 3.39	12.00 ± 2.54	170.17 ± 21.45	92.08 ± 29.40	17.77 ± 4.92
R (400 mg/kg) + LA (120 mg/kg)	25.75 ± 8.22	15.50 ± 7.12	107.25 ± 27.83	40.98 ± 13.99	11.18 ± 3.01

Values are presented as mean ± SEM (n = 6). CMC- Carboxymethylcellulose, R-Resveratrol, LA-Lead acetate, S-Succimer.

APPENDIX V

Table 4.5 Effect of resveratrol on Haematological parameters in Lead-induced Toxicity in Wistar Rats.

Treatments	Pack cell volumes (L/L)	White Blood Cells (L/L)	Platelets (L/L)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
CMC (10 g/L)	3.95 ± 3.53	32.83 ± 5.51	219.50 ± 30.50	25.17 ± 7.29	70.67 ± 6.71	4.60 ± 1.01
LA (120 mg/kg)	0.42 ± 0.03	14.33 ± 4.18	210.50 ± 24.99	22.83 ± 3.90	76.33 ± 3.98	3.00 ± 0.00
LA (120 mg/kg) + S (10 mg/kg)	0.39 ± 0.02	9.98 ± 3.30	261.00 ± 30.51	37.20 ± 4.96	59.60 ± 4.96	4.00 ± 1.56
LA (120 mg/kg) + R (200 mg/kg)	0.50 ± 0.02	12.16 ± 2.87	237.00 ± 19.72	15.80 ± 2.46	83.80 ± 2.25	_
LA (120 mg/kg) + R (400 mg/kg)	0.40 ± 0.02	19.18 ± 5.25	392.33 ± 31.81[a]*	38.00 ± 6.22	49.83 ± 5.93	3.50 ± 0.65
R (400 mg/kg) + LA (120 mg/kg)	0.44 ± 0.02	6.87 ± 0.41	181.67 ± 47.57	18.67 ± 2.91	81.33 ± 2.91	_

Values are presented as mean ± SEM (n = 6). [a] = $p < 0.05$ compared to CMC (10g/L); * = $p < 0.05$ compared to lead acetate (120mg/kg) one way ANOVA followed by tukey *post hoc* test . CMC- Carboxymethylcellulose, R-Resveratrol, LA-Lead acetate, S-Succimer.

APPENDICE VI

Multiple Comparisons

Dependent Variable: Blood lead concentration
Tukey HSD

(I) groups	(J) groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Cmc	LA (N control)	-111.41667*	8.34033	.000	-136.7846	-86.0488
	Succimer	-28.03333*	8.34033	.024	-53.4012	-2.6654
	Resveratrol (200mg/kg)	-27.96667*	8.34033	.024	-53.3346	-2.5988
	Resveratrol (400mg/kg)	-34.26667*	8.34033	.004	-59.6346	-8.8988
	Resveratrol prt (400mg/kg)	-42.36667*	8.34033	.000	-67.7346	-16.9988
LA (N control)	Cmc	111.41667*	8.34033	.000	86.0488	136.7846
	Succimer	83.38333*	8.34033	.000	58.0154	108.7512
	Resveratrol (200mg/kg)	83.45000*	8.34033	.000	58.0821	108.8179
	Resveratrol (400mg/kg)	77.15000*	8.34033	.000	51.7821	102.5179
	Resveratrol prt (400mg/kg)	69.05000*	8.34033	.000	43.6821	94.4179
Succimer	Cmc	28.03333*	8.34033	.024	2.6654	53.4012
	LA (N control)	-83.38333*	8.34033	.000	-108.7512	-58.0154
	Resveratrol (200mg/kg)	.06667	8.34033	1.000	-25.3012	25.4346
	Resveratrol (400mg/kg)	-6.23333	8.34033	.974	-31.6012	19.1346
	Resveratrol prt (400mg/kg)	-14.33333	8.34033	.531	-39.7012	11.0346
Resveratrol (200mg/kg)	Cmc	27.96667*	8.34033	.024	2.5988	53.3346
	LA (N control)	-83.45000*	8.34033	.000	-108.8179	-58.0821
	Succimer	-.06667	8.34033	1.000	-25.4346	25.3012
	Resveratrol (400mg/kg)	-6.30000	8.34033	.973	-31.6679	19.0679
	Resveratrol prt (400mg/kg)	-14.40000	8.34033	.526	-39.7679	10.9679
Resveratrol (400mg/kg)	Cmc	34.26667*	8.34033	.004	8.8988	59.6346
	LA (N control)	-77.15000*	8.34033	.000	-102.5179	-51.7821
	Succimer	6.23333	8.34033	.974	-19.1346	31.6012
	Resveratrol (200mg/kg)	6.30000	8.34033	.973	-19.0679	31.6679
	Resveratrol prt (400mg/kg)	-8.10000	8.34033	.923	-33.4679	17.2679
Resveratrol prt (400mg/kg)	Cmc	42.36667*	8.34033	.000	16.9988	67.7346
	LA (N control)	-69.05000*	8.34033	.000	-94.4179	-43.6821
	Succimer	14.33333	8.34033	.531	-11.0346	39.7012
	Resveratrol (200mg/kg)	14.40000	8.34033	.526	-10.9679	39.7679
	Resveratrol (400mg/kg)	8.10000	8.34033	.923	-17.2679	33.4679

*. The mean difference is significant at the 0.05 level.