THE EFFECTS OF ANTIHYPERTENSIVE DRUGS AND ANTIOXIDANT SUPPLEMENT ON THE DEVELOPMENT AND PROGRESSION OF HYPERTENSION, RENAL OXIDATIVE STRESS AND DAMAGE IN SPONTANEOUSLY HYPERTENSIVE RATS

by

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Thesis submitted in fulfillment of the

requirement for the degree of

Doctor of Philosophy

June 2017

ACKNOWLEDGEMENTS

First and foremost I would like to thank God Almighty for giving me the opportunity and strength to undertake and complete this programme of study.

I am most grateful to my supervisor Professor Dr. K.N.S. Sirajudeen, Department of Chemical Pathology, for all the advice, guidance and encouragement throughout the research project and writing of this thesis. I am also indebted to my co-supervisor Professor Dr. Nik Soriani Yaacob, Department of Chemical Pathology, for her advice and encouragement. Gratitude is also conveyed to Assoc. Prof Dr. Mutum Samarendra for his help in the histopathological work of this project. I would also like to thank our former staff, Professor Dr. H.A. Nadiger, Assoc. Prof Dr. Mummedy Swamy as well as Assoc. Prof Dr. Sunil Gurtu for their advice and guidance.

I wish to express my sincere gratitude to the Head of the Department of Chemical Pathology, lecturers and all laboratory staff for their invaluable assistance during my project work. Special thanks also goes to the Central Research Laboratory, Department of Pathology, Department of Pharmacology, School of Medical Sciences and the Animal Research and Service Centre, USM Kampus Kesihatan for providing excellent research facilities as well as immeasurable help given by their staff. I would also like to thank the past and present management of the School of Medical Sciences as well as USM in general for all the assistance given especially in providing the research grants that helped fund this study.

My deepest gratitude is to my wife, my sons, my mother and my brothers for their love, patience, sacrifices, support and understanding in helping me complete this work. This thesis is dedicated to my late father Mr. Govindasamy a/l Narayanasamy who gave me everything in life and who always emphasized the importance of education.

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4-HNE	4-hydroxynonenal
8-oxo-dG	8-oxo-7,8-dihydrodeoxyguanosine
ACE	angiotensin-converting enzyme
ACEi	angiotensin-converting enzyme inhibitor
ACTH	adrenocorticotropic hormone
ADH	antidiuretic hormone
ADMA	asymmetrical dimethyl-arginine
ALA	alpha-lipoic acid
ALT	alanine transaminase
ALP	alkaline phosphatase
Ang	angiotensin
ANLE	aqueous neem leaves extract
AOE	antioxidant enzymes
ARB	angiotensin receptor blocker
AT1	angiotensin type 1
AT2	angiotensin type 2
BP	blood pressure
BSA	bovine serum albumin
BW	body weight
CAT	catalase
CCB	calcium channel blockers
CDNB	1-chloro-2,4-dinitrobenzene
CHD	coronary heart disease

CKD	chronic kidney disease
CO	cardiac output
COPD	chronic obstructive pulmonary disease
CRF	chronic renal failure
Cu/Zn-SOD	copper/zinc superoxide dismutase
DALYs	disability-adjusted life years
DHLA	dihydrolipoic acid
DNA	deoxyribonucleic acid
DNPH	2,4-dinitrophenyl-hydrazine
DOCA	deoxycorticosterone acetate
DP	diastolic blood pressure
DPPH	1,1-diphenyl-2-picryl hydrazyl
DTNB	5,5’-dithiobis-2-nitrobenzoic acid
EDRF	endothelial derived relaxing factpr
EDTA	ethylenediaminetetra acetic acid
eNOS	endothelial nitric oxide synthase
ESRD	end stage renal failure
FRAP	ferric reduction antioxidant power
GBD	global burden of disease
GFR	glomerular filtration rate
GPx	glutathione peroxidase
GR	glutathione reductase
GSH	reduced glutathione
GSSG	oxidized glutathione
GST	Glutathione-S-transferase

HCl	hydrochloric acid
H & E	hematoxylin & eosin
HOCl	hypochlorous acid
H_2O_2	hydrogen peroxide
ISIAH	inherited stress-induced arterial hypertension
iNOS	inducible nitric oxide synthase
KW	kidney weight
KW/BW	kidney weight / body weight ratio
LDL	low density lipoprotein
LN	N-nitro-L-arginine methyl ester
L-NAME	N-nitro-L-arginine methyl ester
LVH	left ventricular hypertrophy
MAPK	mitogen-activated protein kinases
MDA	malondialdehyde
MPA	metaphosphoric acid
MPO	myeloperoxidase
MT	masson trichrome
NAC	N-acetylcysteine
NADPH	nicotinamide adenine dinucleotide phosphate
NAG	N-acetyl-beta-D-glucosaminidase
NIBP	non-invasive indirect blood pressure
Nm	neem
NO	nitric oxide
NOS	nitric oxide synthase
NOx	sum of both nitrate and nitrite

$ONOO^-$	peroxynitrite anion
PAS	periodic acid schiff
PASM	periodic acid schiff with methenamine silver
PCO	protein carbonyl
PUFA	polyunsaturated fatty acids
PVR	peripheral vascular resistance
RAAS	renin-angiotensin-aldosterone system
RNS	reactive nitrogen species
ROS	reactive oxygen species
SBP	systolic blood pressure
SDS	sodium dodecyl sulphate
SHR	spontaneously hypertensive rat
SHRSP	stroke prone spontaneously hypertensive rat
SNS	sympathetic nervous system
SOD	superoxide dismutase
SVR	systemic vascular resistance
TAS	total antioxidant status
TBA	thiobarbituric acid
TBARS	thiobarbituric acid reactive substances
TCA	trichloroacetic acid
TOD	target organ damage
TNB	5-thio-2-nitrobenzoic acid
VSMC	vascular smooth muscle cells
WKY	Wistar-Kyoto

KESAN UBATAN ANTIHIPERTENSI DAN SUPLEMEN ANTIOKSIDAN KE ATAS PEMBANGUNAN DAN PERKEMBANGAN HIPERTENSI, TEKANAN OKSIDATIF SERTA KEROSAKAN GINJAL DALAM TIKUS HIPERTENSI SECARA SPONTAN

ABSTRAK

Tekanan oksidatif telah dikaitkan dengan pembangunan dan perkembangan hipertensi dan kerosakan organ termasuk ginjal. Walau bagaimanapun peranan yang tepat dan mekanisme yang terlibat tidak jelas kerana kajian yang dilakukan dalam aspek ini adalah terhad terutamanya yang berkaitan dengan ginjal. Kajian ini telah dijalankan untuk mengkaji kesan ubatan antihipertensi tertentu yang diketahui mempunyai ciri antioksidan serta suplemen antioksidan ke atas tekanan oksidatif ginjal semasa pembangunan dan perkembangan hipertensi dan kerosakan ginjal yang terjadi. Kajian telah dilakukan dengan menggunakan tikus hipertensi secara spontan (SHR) serta tikus Wistar-Kyoto (WKY) dan SHR yang diaruh hipertensi disebabkan kekurangan NO melalui pemberian N-nitro-L-arginin metil ester (L-NAME), berbanding dengan tikus normotensif WKY. Kajian fasa pertama terdiri daripada kajian perubahan mengikut masa dalam SBP, parameter morfometrik serta tekanan oksidatif ginjal dalam SHR dari umur 4 minggu sehingga 64 minggu. Ini diikuti dengan kajian dengan tikus WKY, SHR, WKY+L-NAME dan SHR+L-NAME yang melibatkan pemerhatian perubahan dalam SBP, parameter morfometrik serta tekanan oksidatif ginjal pada masa usia 4 minggu usia (pra-hipertensi), usia 16 minggu (hipertensi yang nyata) dan usia 28 minggu (berlaku kerosakan ginjal). Kajian fasa kedua dan ketiga adalah kajian intervensi yang melihat kesan ubat antihipertensi dan suplemen antioksidan ke atas SBP, parameter morfometrik serta tekanan oksidatif ginjal semasa pembangunan dan

perkembangan hipertensi dan kerosakan ginjal. Kajian fasa pertama menunjukkan bahawa SHR menjadi hipertensi pada usia 8 minggu dengan SBP meningkat secara beransur-ansur sehingga usia 64 minggu. SHR mengalami kerosakan ginjal dan tekanan oksidatif ginjal dari umur 24 minggu, yang menjadi semakin teruk secara beransur-ansur sehingga umur 64 minggu sejajar dengan peningkatan SBP. Kajian korelasi menunjukkan terdapat hubungan yang kuat antara tekanan oksidatif ginjal dengan SBP dan kerosakan ginjal. Keputusan juga menunjukkan bahawa tekanan oksidatif berlaku selepas hipertensi terjadi dan bukan sebaliknya. Walabagaimanapun, tekanan oksidatif nampaknya memainkan peranan penting dalam mengekalkan hipertensi serta pembangunan kerosakan ginjal. Aras NOx ginjal di SHR menurun mulai umur 32 minggu. Ini mencadangkan bahawa penurunan NO memainkan peranan yang penting dalam mengekalkan hipertensi. Pengurangan aras NO ginjal yang berlaku bersama dengan kemerosotan hipertensi dan juga selepas tekanan oksidatif dan kerosakan ginjal telah bermula dari minggu 24, menunjukkan bahawa penurunan aras NO berlaku dalam kerosakan ginjal kronik di SHR. Semua hasil ini menunjukkan bahawa dalam SHR, peningkatan tekanan oksidatif dan pengurangan NO mengiringi hipertensi dan menyumbang kepada perkembangan hipertesni serta kerosakan ginjal yang terjadi. Kajian ke atas WKY+L-NAME dan SHR+L-NAME menunjukkan kerosakan fungsi ginjal serta histopatologi pada usia 28 minggu. Ini mengesahkan kesesuaian reka bentuk kajian yang memileh peringkat masa kajian sebagai 4 minggu, 16 minggu dan 28 minggu. Keputusan kajian fasa ini menunjukkan bahawa pada usia 28 minggu, SHR + L-NAME tikus mempunyai SBP, kerosakan ginjal, tekanan oksidatif ginjal dan penurunan aras NOx yang paling tinggi. Ia diikuti oleh SHR dan WKY + L-NAME. Ini menunjukkan bahawa terdapat hubungan yang kuat antara tekanan oksidatif ginjal dan penurunan aras NO dengan hipertensi dan

kerosakan ginjal. Kajian fasa kedua mengesahkan kesan hipotensif ubat antihipertensif clonidine, enalapril dan amlodipine. Enalapril mempunyai kesan hipotensif yang paling tinggi kerana ia dapat mengurangkan SBP di SHR ke tahap normal manakala clonidine dan amlodipine tidak dapat berbuat demikian. Ketiga-tiga ubat menunjukkan keupayaan antioksidan kerana dapat mengurangkan tekanan oksidatif ginjal. Enalapril kelihatan mempunyai kapasiti antioksidan yang paling tinggi manakala amlodipine mempunyai kapasiti terendah. Ketiga-tiga ubat menunjukkan sifat melindung ginjal tetapi dalam darjah yang berbeza, dengan enalapril mempunyai kesan tertinggi dan amlodipine kesan terendah. Enalapril juga dapat memulihkan dengan sepenuhnya aras NOx ginjal dalam ketiga-tiga kumpulan tikus hipertensi. Clonidine hanya mampu untuk meningkatkan dengan ketara tahap NOx dalam SHR+C dan SHR+C+ L-NAME manakala amlodipine tidak dapat meningkatkan aras NOx ginjal dalam mana-mana kumpulan tikus hipertensi. Keputusan ini menunjukkan bahawa mekanisme fisiologi yang terlibat dalam sifat hipotensif dan sifat melindung ginjal enalapril dan clonidine mungkin melibatkan metabolisme NO. Keputusan dalam kajian fasa ini menunjukkan bahawa sifat melindung ginjal dan hipotensif pada ubatan antihipertensif adalah berkait dengan kapasiti antioksidan ubatan itu. Enalapril menunjukkan sifat hipotensif, sifat melindung ginjal serta kapasiti antioksida yang paling tinggi di antara ketiga-tiga ubatan ini. Kajian fasa ketiga menunjukkan bahawa suplemen antioksidan NAC, ALA dan ANLE mempunyai kesan hipotensif tetapi tidak dapat mengurangkan SBP ke aras bawah 140 mm Hg sepanjang kajian ini dalam kesemua kumpulan haiwan hipertensi. NAC dan ALA menunjukkan kesan hipotensif yang sederhana manakala ANLE hanya menunjukkan sedikit kesan hipotensif. Ketiga-tiga suplemen antioksidan menunjukkan sifat melindung ginjal, di mana NAC dan ALA menunjukkan sifat yang sederhana manakala ANLE hanya mempunyai sedikit sifat ini. Ketiga-tiga suplemen antioksidan

juga dapat mengurangkan tekanan oksidatif ginjal di mana NAC kelihatan mempunyai kapasiti antioksidan yang lebih tinggi sedikit daripada ALA manakala ANLE mempunyai kapasiti antioksidan yang paling rendah. NAC juga mampu meningkatkan dengan ketara aras NOx ginjal yang terkurang dalam WKY+NAC+L-NAME dan tikus SHR+NAC+L-NAME, tetapi kedua-dua ALA dan ANLE tidak dapat berbuat demikian. Keputusan ini menunjukkan bahawa mekanisme fisiologi yang terlibat dalam sifat hipotensif dan sifat melindung ginjal oleh NAC mungkin melibatkan metabolisme NO. Secara keseluruhan keputusan yang diperolehi menunjukkan bahawa NAC dan ALA mempunyai sifat hipotensif, sifat melindung ginjal dan antioksidan yang sederhana manakala ANLE hanya mempunyai sedikit sifat-sifat ini. Kesimpulannya, kajian ini menunjukkan bahawa kedua-dua ubat antihipertensi dan suplemen antioksidan yang dikaji, mempunyai sifat hipotensif, melindung ginjal serta sifat antioksidan di mana dapat mengurangkan tekanan oksidatif ginjal. Walau bagaimanapun, ubat-ubatan antihipertensi menunjukkan tahap yang lebih tinggi dalam sifat-sifat ini berbanding dengan suplemen antioksidan.

THE EFFECTS OF ANTIHYPERTENSIVE DRUGS AND ANTIOXIDANT SUPPLEMENT ON THE DEVELOPMENT AND PROGRESSION OF HYPERTENSION, RENAL OXIDATIVE STRESS AND DAMAGE IN SPONTANEOUSLY HYPERTENSIVE RATS

ABSTRACT

Oxidative stress has been implicated in the development and progression of hypertension and subsequent organ damage including the kidneys. However the effect of antihypertensive drugs or antioxidant supplementation on renal oxidative stress during the development and progression of hypertension and the subsequent renal damage has not been well studied. The present study was undertaken to look into the effect of certain antihypertensive drugs with known antioxidant properties as well as antioxidants on renal oxidative stress during the development and progression of hypertension and the subsequent renal damage. The study was performed using spontaneously hypertensive rats (SHR) as well as N-nitro-L-arginine methyl ester (L-NAME) induced nitric oxide (NO) deficient hypertensive Wistar-Kyoto (WKY) and SHR rats in comparison with normotensive WKY rats. The first phase study consisted of a time course study on changes in systolic blood pressure (SBP), body morphometric parameters and renal oxidative stress status in SHR from the age of 4 weeks until 64 weeks, followed by the study on L-NAME induced NO deficient hypertensive WKY and SHR rats involving observation of these parameters at the time points of 4 weeks of age (prehypertension), 16 weeks of age (established hypertension) and 28 weeks of age (occurrence of renal damage). The second and third phase studies were intervention based studies which looked into the effect of antihypertensive drugs

and antioxidants on these parameters during the development and progression of hypertension and the subsequent renal damage. The first phase studies showed that SHR became hypertensive by the age of 8 weeks, with the SBP increasing gradually until 64 weeks of age. SHR developed renal damage and renal oxidative stress from the age of 24 weeks, which worsened gradually until the age of 64 weeks in line with increasing hypertension. Correlation studies suggest a strong relationship between renal oxidative stress with SBP and renal damage. The results also indicate that oxidative stress is a consequence of hypertension and not a cause of it, however it appears to play a prominent role in the maintenance of hypertension and development of renal damage. Renal NOx levels in the SHR decreased from the age of 32 weeks, which occurred together with worsening hypertension and also after oxidative stress and renal damage had commenced from week 24, indicating that the decrease in NO levels occurs as the chronic renal damage in SHR progresses. This suggests that in the SHR, increased renal oxidative stress and reduced NO bioavailability accompanies hypertension and contributes to its maintenance and progressive damage of the kidneys. Studies on the L-NAME induced hypertensive WKY and SHR rats showed that at 28 weeks of age, SHR+L-NAME rats had the highest SBP, renal damage, renal oxidative stress and reduced NOx levels, followed by SHR and WKY+L-NAME rats. This suggests a strong relationship between renal oxidative stress and reduced NO bioavailability with hypertension and renal damage. The second phase study confirmed the hypotensive effect of clonidine, enalapril and amlodipine. Enalapril had the greatest hypotensive effect as it was able to reduce SBP in SHR to normotensive levels while clonidine and amlodipine were not able to. All three drugs showed antioxidant capabilities as they were able to reduce renal oxidative stress. Enalapril appeared to have the highest antioxidant capacity with amlodipine having the least. All three drugs

showed renoprotective properties with enalapril having the highest renoprotective effect and amlodipine having the least effect. Enalapril was able to fully restore the reduced renal NOx levels in all three hypertensive groups. Clonidine was only able to significantly increase NOx levels in SHR+C and SHR+C+L-NAME rats while amlodipine was not able to increase renal NOx levels in any of the hypertensive animal groups. These results suggest that the physiological mechanisms involved in the hypotensive and renoprotective properties of enalapril and clonidine might involve NO metabolism. Results from this phase of study suggest that the renoprotective and hypotensive properties of these antihypertensive drugs are associated with its antioxidant capacity, with enalapril showing the greatest hypotensive and renoprotective property as well as antioxidant capacity. The third phase study showed that the antioxidant supplements N-acetylcysteine (NAC), alpha-lipoic acid (ALA) and aqueous neem leaves extract (ANLE) had hypotensive effect but were unable to reduce SBP to levels below 140 mm Hg in any of the hypertensive animal groups. NAC and ALA showed moderate hypotensive effect while ANLE only showed slight hypotensive effect. All three supplements showed significant renoprotective property, whereby NAC and ALA showed moderate renoprotective property while ANLE only had slight renoprotective property. All three supplements were able to reduce renal oxidative stress whereby NAC appeared to have slightly higher effect than ALA with ANLE having the lowest effect. NAC was also able to significantly increase the reduced renal NOx levels in WKY+NAC+L-NAME and SHR+NAC+L-NAME rats, while both ALA and ANLE did not increase the depressed NOx levels in any of the hypertensive rat groups. This result suggests that the physiological mechanisms involved in the hypotensive and renoprotective properties of NAC might involve NO metabolism. Overall the results obtained suggest that NAC and ALA have moderate

hypotensive, renoprotective and antioxidant properties while ANLE has only slight degree of these properties. In conclusion, this study showed that both the antihypertensive drugs and the antioxidant supplements that were investigated , had hypotensive, renoprotective as well as antioxidant properties. However the antihypertensive drugs showed a much higher degree of these properties compared to the antioxidant supplements.

CHAPTER 1

GENERAL INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The cardiovascular system of the body plays a crucial role in health as it sustains the metabolic demands of all the organs through the pumping action of the heart and the vascular system for generating and maintaining an adequate blood supply to all the tissues. As such, disorders of the cardiovascular system represent a major health concern as it leads to further health problems. Among the cardiovascular disorders, hypertension, a chronic health condition in which systemic arterial pressure is persistently elevated, has emerged as a global public health problem due to its high prevalence which in 2014 was about 22 % of the adult population aged 18 years and above (WHO, 2015). Its prevalence also rises with age (Staessen *et al*., 2003; Frans *et al*.,2008; Amal *et al*., 2011) whereby worldwide its prevalence for adults aged 25 years and above, was 40 % in 2008 (WHO, 2013). At present, more than a billion adults in the world have hypertension and this figure is predicted to increase 50-60 % to about 1.56 billion by 2025 with greater number in the developing countries (Kearney *et al*., 2005; WHO, 2015). In Malaysia, its prevalence is even higher at about 32.7% whereby approximately 5.8 million adults above the age of 18 have hypertension whereas among adults aged 30 years and above, the prevalence is 43.5 % (Ministry of Health, 2011).

Hypertension if not adequately controlled can lead to damage of various organs resulting in serious health problems such as stroke, myocardial infarction, cardiac failure, dementia, renal failure and blindness, making it a significant contributor to global morbidity and mortality. In 2010, hypertension was estimated to

have caused 9.4 million deaths, making it one of the leading physiological risk factors to which 13 % of global deaths are attributed. (Lawes *et al*., 2006; Lim *et al*., 2012). The Global Burden of Disease Study (GBD), which quantifies the burden of disease in disability-adjusted life years (DALYs), a time-based measure that combines years of life lost due to morbidity and premature mortality, has ranked hypertension as the leading single risk factor for GDB in 2010. Hypertension was found to contribute to about 7% of disease burden worldwide as measured in DALYs, causing it to have a negative impact on the quality of life. Approximately two-thirds of this attributable disease burden occurred in the developing countries, mostly in the 45-69 years old age group (Bromfield and Muntner, 2013; Lim *et al*., 2012; WHO, 2014).

The economic burden of hypertension is also enormous as it extends far beyond that related to its direct treatment alone. It is estimated that over a ten year period, hypertension may cost nearly US $1 trillion in global health direct costs (Gaziano *et al*.,2009). In Malaysia, the Ministry of Health spent about RM380.9 million on antihypertensive medication alone in 2011. Studies in Malaysia have shown that the direct cost of treating hypertension increased as hypertension worsened and the cost of treating hypertension is much higher depending on whether one or more co-morbidities like diabetes and hyperlipidemia exist together with hypertension (Alefan *et al*.,2009; Azimatun *et al*., 2014). As such, the actual cost of treating hypertension is greatly increased by the cost of treating complications of hypertension like heart failure, myocardiac infarction, stroke and renal disease (MOH, 2011). The indirect costs of hypertension include the loss of productivity due to absenteeism, illness and death. It is estimated that globally the indirect costs amount to about US $3.6 trillion (Gaziano *et al*.,2009). It is obvious then that hypertension is a costly burden that requires our utmost attention.

Hypertension is generally classified as either primary, which may develop as a result of environmental and or genetic causes, or secondary, which has multiple etiologies, including renal, vascular and endocrine causes. Primary or essential hypertension accounts for about 95 % of all cases of hypertension (Beevers *et al*., 2001). However the exact cause(s) or mechanisms involved in its pathogenesis have not been elucidated (Carretero and Oparil, 2000). While various pathophysiologic factors have been implicated in the genesis of essential hypertension, the kidney, through intrinsic mechanisms, is strongly believed to play a key role, giving rise to the phrase 'hypertension follows the kidney' (Oparil *et al*., 2003; Guyton, 1991; Crowley and Coffman, 2014). This aspect of 'hypertension follows the kidney' has been supported by transplantation studies (Coffman *et al*., 1989; Rettig, 1993). At the same time, the kidney is also one of the main targets of organ damage when hypertension is not controlled as it leads to chronic kidney disease and eventually end-stage renal disease (ESRD). Hypertension is believed to account for approximately 30 % of cases of ESRD (Glassock, 2004; Jamerson and Townsend, 2011). As such, while hypertension is a multiorgan disease, the kidneys are believed to play a central role in the development of hypertension and at the same time a target of hypertension-induced damage (Touyz, 2012). All this points to the importance of research in the kidneys itself when investigating the mechanisms involved in the pathogenesis and progression of hypertension as well as kidney damage due to hypertension.

One of the mechanisms implicated in the pathogenesis and progression of hypertension including organ damage, is free radical mediated oxidative damage (Touyz, 2000; Wilcox, 2002). Free radicals and their metabolites, reactive oxygen species (ROS), are constantly formed in the body by several mechanisms, involving both endogenous and environmental factors. These substances being reactive, can cause oxidative damage to biological molecules. Antioxidants are substances that

significantly delay or inhibit the oxidation of substrates (Halliwell and Gutteridge, 1992). The body possesses antioxidant systems that are very important to protect cellular components from free radical induced damage. Under physiologic conditions, ROS produced in the course of metabolism are contained by the body's antioxidant defence mechanisms. When these defence mechanisms are inadequate, either due to increased ROS production or diminished antioxidant levels, oxidative stress occurs. Oxidative stress, the state in which cells are exposed to excessive levels of molecular oxygen or ROS, leads to damage of biological molecules such as lipids, proteins, carbohydrates and DNA. This in turn can inflict tissue injury and dysfunction (Lunec, 1990; Halliwell, 1994). Several reports have documented that hypertension is associated with increased free radical production as well as reduction of antioxidant capacity (Tse *et al*.,1994; Russo *et al*.,1998; Pedro-Bolet *et al*.,2000). However these studies have not been comprehensive enough as they did not examine the development of hypertension in a detailed time course manner in relation to all the important antioxidants and related metabolites. These studies also did not focus much on the involvement of the kidney.

The present treatment for essential hypertension involves initial life style modifications which if not effective is followed by pharmacological treatment with antihypertensive drugs to control the blood pressure within normal limits so as to prevent end organ damage. However current data show that most people with hypertension worldwide are not effectively treated and controlled to the recommended blood pressure levels (Kearney *et al*., 2004; Israili *et al*.,2007; Messerli *et al*., 2007). In the United States less than 50 % of hypertensives on medication have their blood pressure reduced to normal levels (Crowley and Coffman, 2014). In Malaysia, only 35 % of patients on medication have their blood pressure controlled within normal limits (MOH, 2011). Overall, even though newer classes of antihypertensive drugs

have been introduced, the number of people with uncontrolled hypertension and subsequent end organ damage, has continued to rise (Chobanian, 2009). In addition to this, the various side or adverse effects of antihypertensive drugs affect its tolerability as it impacts negatively on the quality of life (Carvalho, 2013). Based on this, various alternative or complementary therapies are being looked into for the management of hypertension. In this regard, since oxidative stress has been implicated in the development and progression of hypertension, supplementation with antioxidants has also been looked into for the treatment and management of hypertension (Wen *et al*.,1996; Akpaffiong and Addison, 1998). This is especially so as studies have shown that mobilization of antioxidants occurs in response to oxidative stress which reflects a dynamic process whereby dietary antioxidant supplementation might exert a significant influence (Nabil, 2001). In this respect, various research on antioxidant levels and effect of antioxidant supplementation in hypertension have been undertaken but the results obtained are conflicting as some studies showed that supplementation was beneficial (Park *et al*.,2002; Chen *et al.*,2000) whereas in others it was not (Kim *et al*.,2002; Stephens *et al*.,1996). Even though some studies have shown that supplementation with antioxidants reduce blood pressure and certain oxidative stress parameters, the studies concerned did not look extensively into the role and biochemical mechanisms of oxidative stress as well as the antioxidant defense systems involved in the kidney. Based on this and also the fact that it is still not clear whether it is increased free-radical generation or a reduced defence against these radicals that contributes to oxidative stress in the development and maintenance of hypertension, further studies involving supplementation of antioxidants in hypertension are needed to provide more information. This is especially so for understanding the role of oxidative stress in the kidney as studies in this area have been limited.

In relation to the role of oxidative stress in hypertension, some studies have shown that certain antihypertensive drugs have antioxidative properties, suggesting that the therapeutic benefit of these drugs including renoprotection could be in part due to their antioxidant properties whereby there is inhibition of free radical production. These studies involving both human and animal models including the spontaneously hypertensive rat (SHR), have demonstrated that certain groups of antihypertensive drugs lower blood pressure as well as cause changes in the oxidative status (Mak *et al*.,1992; Wiemer *et al*.,1997; Mantle *et al*.,2000; Bayorh *et al*.,2003). However the studies concerned were not comprehensive as no in-depth study have been carried out on the effect of these antihypertensive drug treatment on the antioxidant mechanisms involved in the kidney before and during hypertension as well as after kidney damage occurs. As such the biochemical mechanisms by which these antihypertensive drugs might inhibit oxidative stress in the kidneys is not well known. Further studies are needed to clarify whether these antihypertensive drugs function by affecting the antioxidant defence mechanisms in the kidneys or just primarily correct the altered mechanical forces that cause structural changes in the kidney.

Overall, the role of oxidative stress and related protective mechanisms in the kidney in the development, progression and subsequent kidney damage as well as how it is affected by antihypertensive drugs and antioxidant supplementation is still not clear and fully understood. As such, this study using the SHR, aims to provide answers by examining the renal oxidant/antioxidant status during the development and progression of hypertension including renal damage as well as the effect antihypertensive drugs and antioxidant supplements have on it.

1.2 REVIEW OF LITERATURE

1.2.1 Hypertension

Pressure is required to move blood throughout the circulatory system for the various needs and functions of the body. This pressure is primarily determined by the cardiac output of the heart and the resistance of the blood vessels, mainly the peripheral vascular system, towards the flow of blood. The resultant blood pressure (BP) is the force exerted by circulating blood on the walls of the arteries and veins. Venous pressure however is very low, as such BP is generally equated to arterial pressure (Ram, 2014).

BP measurement is given in mm mercury (Hg) as two values whereby the first value is the systolic pressure, followed by the second value, the diastolic pressure. Systolic blood pressure (SBP) is the peak pressure in the arteries, which occurs when the ventricles are contracting to pump out blood into the systemic arterial circulation. Diastolic blood pressure (DP) is the residual minimum pressure left in the arterial system when the ventricles relax. Normal BP at rest is within the range of 100–140 mm Hg systolic and 60–90 mm Hg diastolic (Ram, 2014).

Hypertension is defined as persistently elevated blood pressure whereby the SBP is greater than 140 mm Hg and/or the DP is greater than 90 mm Hg. According to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7, USA), the stratified classification of BP for adults aged 18 years or older is as in Table 1.1 :

Table 1.1 : Classification of blood pressure for adults aged 18 and above. (Chobanion *et al*., 2003)

Category	Systolic (mmHg)		Diastolic (mmHg)
Optimal	< 120	and	< 80
Prehypertension	120-139	or	80-89
Hypertension Stage 1	140-159	or	90-99
Hypertension Stage 2	≥160	or	≥100

Compared to previous classifications, the above classification creates the new category of prehypertension which combines the previous above optimal normal and high normal ranges of BP (Chobanion *et al*., 2003). This new classification indicates the importance of the prehypertensive category where the present focus is for both research and therapeutic measures so as to prevent the occurence of hypertension (Svetkey, 2005).

The usual BP measurement indicates both systolic and diastolic pressure values, however it is SBP values that are being given greater importance now. Previously DP was believed to be the better indicator of health risk when compared to SBP. This changed when the Framingham Study showed that SBP had greater predictive value than DP for cardiovascular disease development for all ages and both gender (Kannel ,1996). This led the National High Blood Pressure Education Program of the United States to recommend that SBP be ascribed a more important role in the diagnosis and treatment of hypertension (Izzo *et al*, 2000). Subsequent studies by other researches showed that SBP was the best indicator of cardiovascular risk especially after 50 years of age (Vardan and Mookherjee, 2000). Further studies by Hozawa *et al* (2000) and

Benetos *et al* (2001) strongly suggest that the prognosis of hypertension should be based on SBP and not DP. Related to this is the finding that SBP is more important for determining renal damage when compared to DP (Klag *et al*., 1996). As such, researchers currently performing studies on hypertension tend to focus more on SBP than DP.

Hypertension is termed the 'silent killer' because it usually does not cause symptoms initially, making people unaware that they have it. It can progress insidiously undetected leading to more serious complications involving organ damage such as heart disease, coronary artery disease, stroke, peripheral artery disease, blindness and chronic kidney disease. All these complications cause great damage and increase the risk of death (WHO, 2013). This makes it very important that hypertension is detected early so that prompt treatment can be initiated to control and bring down the elevated BP to an acceptable level.

In terms of etiology, hypertension is classified as either primary (essential) hypertension or secondary hypertension. Primary hypertension, defined as high blood pressure with no obvious underlying cause, accounts for about 90–95% of all cases. The remaining 5–10% of cases are categorized as secondary hypertension, defined as hypertension that has arisen secondary to an identifiable cause such as chronic kidney disease, narrowing of the aorta or kidney arteries or an endocrine disorder (Chobanion *et al*., 2003). Between these two categories, it is primary or essential hypertension that poses the much greater challenge in the medical field due to its unknown etiology as well as much higher occurrence.

Essential hypertension is considered a heterogenous disorder with different patients having different causal factors that lead to abnormally increased BP. While the exact cause(s) of essential hypertension is unknown, various risk factors have

been identified as contributors towards it. These risk factors can be generally classified as inherited, behavioural or metabolic risk factors as shown in Table 1.2 (Ford and Cooper, 1991; Whelton *et al*.,2002 ; Chobanion *et al*., 2003 ; Yadav *et al*.,2008; Loh *et al*.,2013). While the inherited risk factors are unmodifiable, the behavioural and metabolic risk factors are modifiable, enabling them to be lessened or eliminated so that the risk of developing hypertension is greatly reduced or averted (Whelton *et al*.,2002; Chobanian *et al*.,2003).

Table 1.2 : Risk Factors for Essential Hypertension

Category	Risk Factor
Inherited	Genetics (Hereditary / Family history) Ethnicity (South Asians, Africans) Age (increasing) Gender (male)
Behavioural	Physical inactivity Smoking Alcohol abuse Unhealthy diet - high sodium, lipids - low potassium High Stress Chronic lack of sleep
Metabolic	Overweight / Obesity Hyperlipidemia Diabetes / Impaired glucose tolerance

1.2.1.1 Pathophysiology of Essential Hypertension

Blood pressure is the force of blood exerted against the walls of arteries during its circulation from the heart throughout the body. It is the product of cardiac output (CO) and systemic vascular resistance (SVR) in which the balance between them determines the actual blood pressure that is produced (Giles *et. al*., 2009; Foex and Sear, 2004). CO is regulated by heart rate, primarily controlled by the autonomic nervous system, and stroke volume which is affected by the volume of circulating

blood. The SVR is the resistance to blood flow caused by all of the systemic vasculature except the pulmonary vasculature. SVR is also usually referred as peripheral vascular resistance (PVR) as vascular resistance is deemed as mainly caused by the peripheral blood vessels (Foex and Sear, 2004). Overall, these two primary determinants are in turn determined by neural, humoral and local mechanisms of cardiovascular and renal function control as shown in Figure 1.1. The detailed complex interaction of these physiologic and other environmental factors in the control and regulation of BP are as displayed in Figure 1.2. As BP can be affected by any of these factors, it follows that hypertension can also be caused by abnormality in any one or a multitude of these factors. However pinpointing the exact cause is difficult because BP is an integrated value determined by variable contributions from all these factors. It is also very hard to determine primary or causal factors for abnormally increased BP from those responses that are secondary to BP changes (Vikrant and Tiwan, 2001; Silva, 2006).

Even though the exact pathophysiology involved in the development and progression of essential hypertension is still unknown, various pathophysiologic mechanisms have been postulated and implicated for it. As a summary, the main pathophysiologic factors believed to play a role in the development and maintenance of essential hypertension can be grouped under neurohormonal mechanisms, dietary factors, vascular factors, cellular mechanisms and other factors such as inflammation, psychosocial stress and also novel factors such as oxidative stress as shown in Table 1.3. Among these various factors, the main factors that have been focussed and researched on are the sympathetic nervous system (SNS), the renin- angiotensin-aldosterone system (RAAS), sodium intake and metabolism and vascular changes. Many of these factors are regulated by or involves the kidney, giving it a central role

as one of the main drivers in the pathogenesis and progression of hypertension (Navar, 2005).

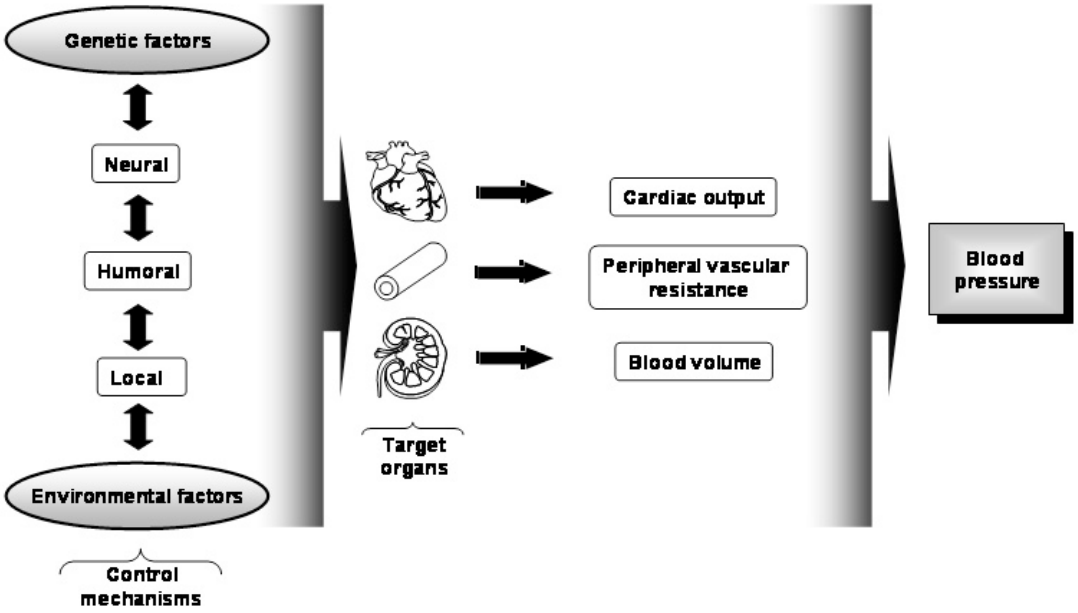

Figure 1.1 Some of the factors involved in the control of blood pressure (adapted from Silva, 2006)

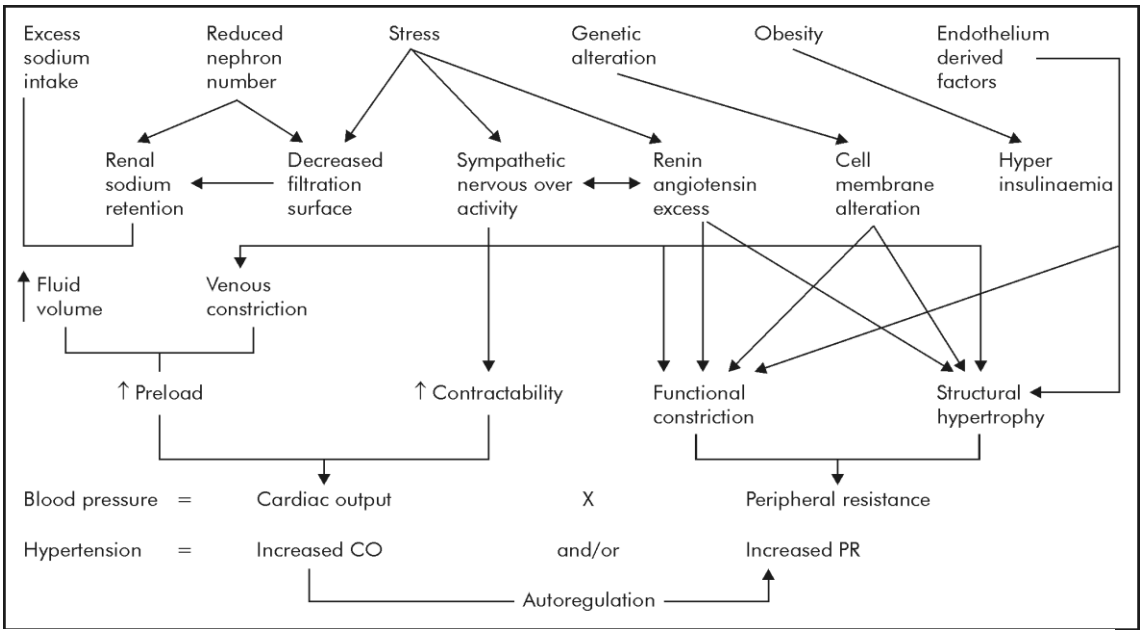

Figure 1.2 : Some of the factors involved in the control of blood pressure that affect the basic equation : blood pressure – cardiac output x peripheral resistance. (adapted from Vikrant and Tiwari, 2001)

Table 1.3 : Pathophysiological factors that play a role in the development and maintenance of hypertension
(adapted from Acelajado *et al*., 2013)

Pathophysiologic Factor	Mechanism (Increased or decreased activity)
Neurohormonal Mechanisms	
SNS activity	↑
RAAS	↑
Production of sodium retaining hormones	↑
Production and expression of vasoconstrictors	↑
Production and expression of vasodilators	↓
Kallikrein-kinin system activity	↓
Dietary Factors	
Sodium intake	↑
Potassium and calcium intake	↓
Vascular Factors	
Peripheral resistance	↑
Vascular stiffness	↑
Endothelial dysfunction	↑
Cellular Mechanisms	
Cellular ion transport	↑ or ↓
Adrenergic receptor activity	↑ or ↓
Others	
Inflammation	↑
Psychosocial stress	↑
Oxidative stress	↑

1.2.1.1 (a) Sympathetic Nervous System

The sympathetic nervous system (SNS) is part of the autonomic nervous system which also includes the parasympathetic nervous system. The SNS provides widespread direct and indirect control of cardiac and vascular function, innervating the brain, heart, blood vessels, adrenal gland and kidneys. The SNS thus connects the brain, heart, blood vessels and kidneys, each of which plays an important role in the regulation of blood pressure. Under normal conditions, the SNS plays a major physiologic role in rapid control of BP whereby it responds appropriately to increases and decreases in BP via baroreflex and chemoreflex receptor pathways at both peripheral and central levels. In addition, the renal sympathetic nerves are believed to

play an important role in long-term BP control by affecting various renal related metabolic processes involved in BP homeostasis (Lohmeier, 2001; Schlaich *et al.*,2009).

Studies have indicated that increased SNS activity contributes to both the development and the maintenance of hypertension (Smith *et al*.,2004). This increased SNS activity is believed to result in the stimulation of the heart, peripheral vasculature and kidneys, causing increased cardiac output, increased vascular resistance and fluid retention (Mark, 1996; Grassi et al.., 1998; Mancia *et al*.,1997). In relation to this, increased activity of the renal sympathetic nerves has been identified as a major contributor to the complex pathophysiology of hypertension (DiBona and Sawin, 2004; Grisk and Rettig, 2004). Even though the exact cause(s) of increased SNS activity has not been identified, several factors or mechanisms have been postulated for it (Mancia and Grassi, 2014). This includes baroreflex dysfunction (Grassi *et al*., 1998), chemoreceptor stimulation (Trzebski, 1992), stimulation of afferent sympathetic nerve fibers (DiBona and Kopp, 1995; Xu *et al*.,2014), increased insulin and leptin levels (Mark *et al*.,1999) and increased angiotensin II (Saino *et al*., 2000).

1.2.1.1 (b) Renin-Angiotensin-Aldosterone System (RAAS)

The RAAS is one of the major hormonal systems for the regulation of blood pressure. It does this by controlling the normal effective circulating blood volume and systemic vascular resistance. In this system, renin is synthesized as an inactive precursor, prorenin, by the the juxtaglomerular (JG) cells that line the afferent arteriole of the renal glomerulus. It is stored there and activated before being secreted into the renal and then the systemic circulation when stimulated in response to a fall in renal glomerular perfusion pressure, reduced concentration of sodium chloride in renal tubular fluid or increased activity of the SNS (Beevers *et al*., 2001; Atlas, 2007).

Control of renin secretion is the primary mechanism by which the RAAS regulates BP and volume homeostasis. It is the key determinant of the activity of the RAAS. The secreted renin in the plasma then regulates the initial, rate-limiting step of the RAAS by cleaving the substrate angiotensinogen, released by the liver, to form the inactive decapeptide angiotensin I (Ang I). Ang I is in turn cleaved by angiotensin converting enzyme (ACE) to form the active octapeptide angiotensin II (Ang II). ACE is a membrane- bound enzyme synthesized by various cells including vascular endothelial cells throughout the blood circulation (Acelajado *et al*., 2013; Atlas, 2007). Ang II, the primary active product of the RAAS, acts via receptors, mainly the type 1 (AT1) receptor and to a much less extend the type 2 (AT2) receptor which is expressed at low levels in adults. Most of the established physiological and pathophysiological effects of Ang II are mediated through the AT1 receptor. The AT1 receptor when activated causes vasoconstriction, aldosterone and antidiuretic hormone release, central sympathetic activation, renal salt and water retention and other actions, that cause systemic vasoconstriction and increased blood volume (Fig 1.3). These actions induce elevation of blood pressure (Acelajado *et al*., 2013; Atlas, 2007).

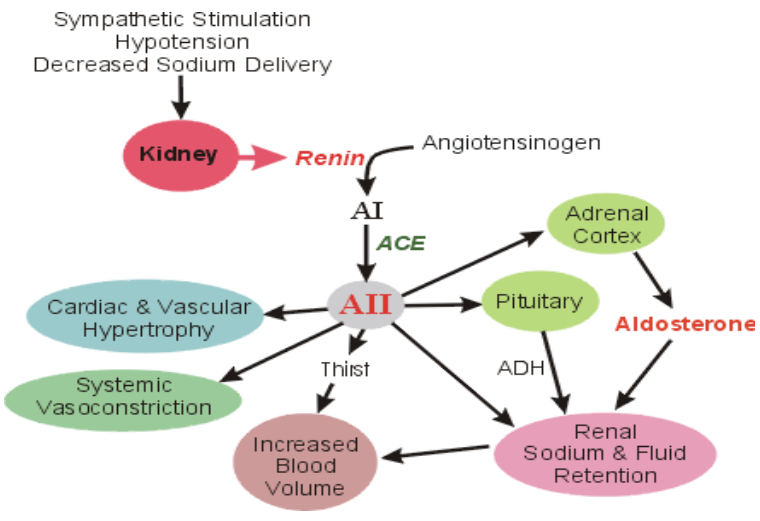

Figure 1.3 – The RAAS is responsible for the production of the BP regulating hormone Ang II (adapted from Bhuyan and Mugesh, 2011)

Besides the existence of the established systemic RAAS, studies have also indicated the presence of local tissue specific renin-angiotensin system (RAS) for both the generation and action of Ang II in various organs including the kidney (Lavoie and Sigmund, 2003). The intrarenal RAS is hypothesized to regulate systemic BP and aspects of renal function such as blood flow and sodium reabsorption (Navar *et al*., 1997; Kobori *et al*., 2007). Based on these findings, the present prevailing concept is that the RAAS functions both as a circulating system and as a tissue paracrine / autocrine system (Atlas, 2007).

Under normal circumstances, the RAAS maintains salt and water homeostasis and BP regulation. However abnormal activation of the RAAS leads to aberrant fluid and electrolyte metabolism, increased vasoconstriction and elevated BP (Conlin *et al*., 1997; Schlaich *et al*., 2009). Studies have shown that this abnormal activation of the RAAS results in increased synthesis of Ang II at systemic and renal tissue level (Silva, 2006). This dysregulation of the RAAS is believed to be involved in the pathogenesis of hypertension (Atlas, 2007).

1.2.1.1 (c) Sodium intake and fluid balance

Epidemiology studies strongly suggest that increased sodium intake can lead to the development of essential hypertension as it is seen primarily in societies with average sodium intakes above 100 meq/day (2.3 g) but rare in societies with average sodium intake of less than 50 meq/day (1.2 g) (Adrogue and Madias, 2007; Elliot *at al*.,1996; Jones, 2004). This also suggests that a threshold level of sodium intake is required for the development of essential hypertension. Studies have also shown that reducing sodium intake decreases BP by up to 8-10 mm Hg (Cook *et al*., 2007; Pimenta *et al*.,2009). Chloride, the accompanying anion in salt, also seems to be important in the pathogenesis of essential hypertension as studies which used other

combinations of anions with sodium or chloride with other cations instead of sodium chloride, did not produce the same results (Kurtz *et al*.,1987).

Sodium chloride is a primary determinant of extracelluar fluid volume. Its level in the body is regulated by the kidneys, which in association with other functions, determines the blood and plasma volume. This in turn affect the cardiac stroke volume and subsequently the BP. The kidneys respond to variations in dietary sodium intake by dynamic regulation of sodium and water excretion so that the extracelluar fluid volume is maintained for enabling normal BP. In relation to this, impaired sodium excretion leading to increased extracelluar fluid volume, has been a hallmark of hypertension (Krzesinski and Cohen, 2007).

While the exact mechanisms by which this salt sensitivity where increased salt intake leads to hypertension, has not been elucidated, several factors and mechanisms have been suggested. Decline in renal function due to age has been suggested for the inability of the kidney to excrete sodium, especially in the elderly (Acelajado and Oparil, 2009). Increased levels of endogenous sodium pump inhibitors in the kidney are said to play a role in developing salt sensitivity leading to hypertension (Blaustein, 1996; Anderson *et al*., 2008). Other studies have indicated that increased dietary sodium causes significant changes in vascular tone and structure which results in increased peripheral vasoconstriction and eventually hypertension (Sanders, 2009).

1.2.1.1 (d) Vascular changes

Even though a number of organ systems, especially the kidneys, play important roles in the pathophysiology of essential hypertension, the present view is that it is considered a disease of vessels i.e. vasculopathy (Touyz, 2012). It is clear that alterations in vascular structure, mechanical properties and function are paramount, culminating in increased peripheral vascular resistance (PVR) which is

considered the hallmark of hypertension (Staessen *et al*., 2003). This vasculopathy basically occurs in the small arteries and arterioles which are considered the main vascular resistance vessels in hypertension. The vascular changes that occurs involves structural remodeling, increased stiffness and reduced distensibility, endothelial dysfunction and inflammation (Oparil *et al*., 2003; Touyz, 2012).

Vascular remodeling of the resistance vessels can involve hypertrophic remodeling as a result of smooth muscle cell hypertrophy in the media of the vessel, hyperplasia leading to the growth of additional cells within the media as well as deposition of extracellular matrix elements (collagen, fibronectin and reductin) in the media (Intengan *et al*., 1999; Intengan *et al*., 2000). Besides hypertrophic remodeling, the smaller resistance vessels can also undergo inward eutrophic remodeling (Schiffrin *et al*., 2000). The end result of both types of remodeling is a reduced lumen diameter of the vessel, resulting in increased resistance to blood flow in these vessels with overall increase in PVR (Mulvany, 1999).

Vascular rarefaction, the decrease in the number of small arterioles, is another form of vascular remodeling. This phenomena also increases PVR and contributes to hypertension. It is thought that vascular rarefaction is initially a temporary functional change to help protect the capillary beds from the mechanical stress that accompanies the elevated BP but over time it may become permanent (Serne *et al.*, 2001).

Cellular processes involved in these vascular changes include vascular smooth muscle cell growth/apoptosis, altered endothelial cell function, fibrosis, hypercontractivity and calcification (Touyz, 2012). All these vascular changes are thought to be initially adaptive processes to help the body cope with the elevated BP but over time they become maladaptive and contribute directly to hypertension and further complications (Touyz, 2012).

Studies have shown that the vascular changes that occur in hypertension are present even in persons with prehypertension, suggesting that vascular remodeling antedates the development of actual hypertension. This has resulted in great interest and extensive research as it raises the question as to what extent resistance vessel structure plays a direct role in setting the BP and in the pathogenesis of essential hypertension (Oparil *et al*., 2003).

1.2.1.2 Consequences and Complications of Hypertension

Hypertension that is not adequately treated leads to complications mainly due to the vascular damage that has occurred. This in turn causes damage to targeted organs i.e. the heart, brain, eyes and kidneys and increasing the risk of morbidity and mortality. In general, the degree of hypertensive target organ damage (TOD) is proportional to the duration and severity of hypertension. Usually the presence of any given form of TOD signals the likelihood that other major target organs have also been damaged, clearly increasing the risk for overall morbidity and mortality (Izzo *et al*., 2013). The usual progression of TOD is from a subclinical phase with few symptoms (oligo symptomatic) to the clinical phase which has clear symptoms and finally to end-stage disease where it is poly symptomatic (Messerli *et al*., 2007) as shown in Fig 1.4. Overall the various derangements and TOD that can occur due to complications of hypertension are as summarized in Fig 1.5 (Schmeider, 2010).

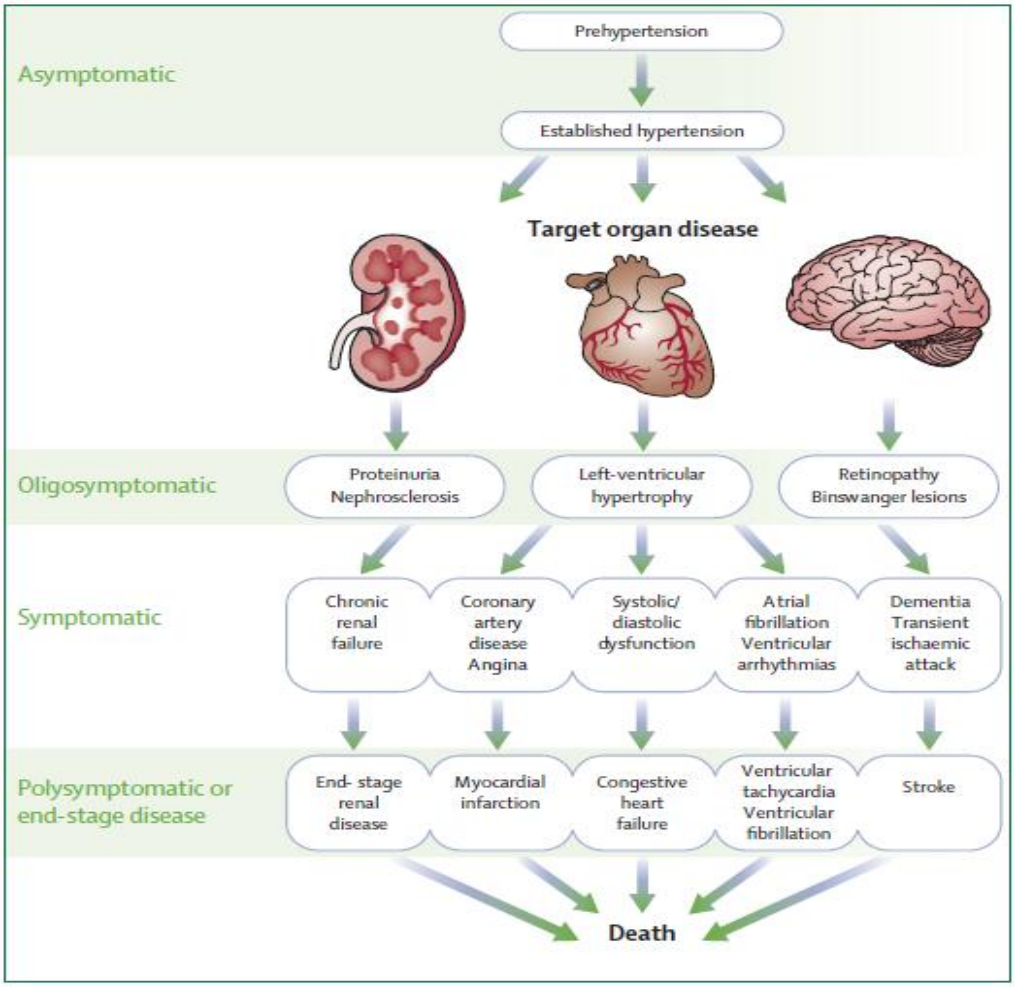

Figure. 1.4 : Range of hypertensive cardiovascular disease from prehypertension to target-organ damage and end-stage disease
(adapted from Messerli *et al*., 2007)

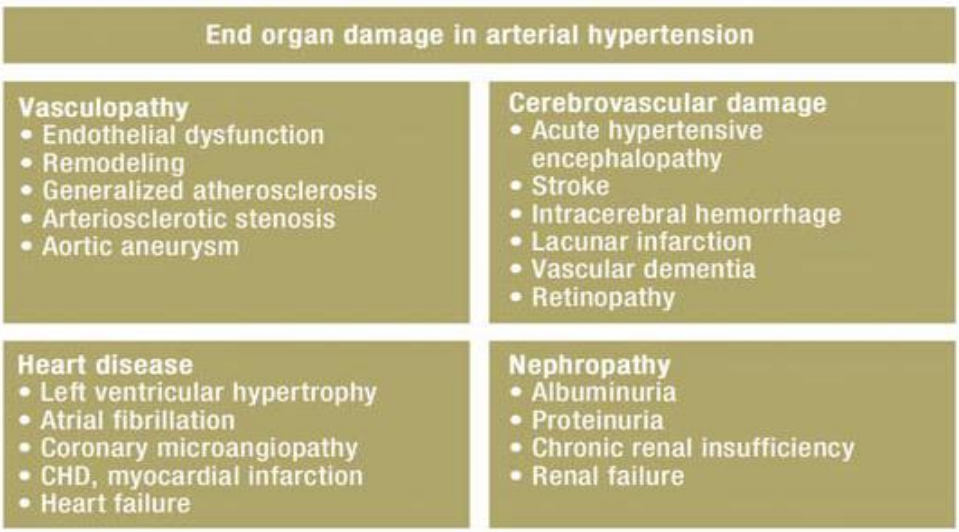

Figure. 1.5 : End organ damage in arterial hypertension
(adapted from Schmeider, 2010)

1.2.1.3 Renal Damage

Hypertension is one of the major factors contributing to kidney damage in the form of chronic kidney disease (CKD) and further complications. It is the second main cause of end-stage renal failure (ESRF) after diabetes mellitus, accounting for about 30% of cases (Glassock, 2004). Equally important is that the prevalence of both CKD and ESRF has been rising over the years (Platinga *et al*., 2009). Studies have shown that the risk of ESRF is directly linked to BP level (Klag *et al*., 1996). It was found that SBP of 140 to 159 mm Hg significantly increased risk for ESRF or death by 38 % compared with those below 130 mm Hg. Also every 10 mm Hg rise in baseline SBP, significantly increased the risk for ESRF or death by 6.7 % (Bakris *et al*., 2003).

In hypertension, the extent of renal damage is proportional to the degree of arterial pressure exposure of renal microvasculature. Renal injury occurs due to vascular damage that causes arteriosclerosis especially involving the preglomerular vessels (Sommers and Melamed, 1990). This leads to increased renal vascular resistance that causes elevation of intraglomerular capillary pressure. This resultant glomerular hypertension causes glomerular capillary stretching, endothelial damage and elevated glomerular protein filtration, leading to glomerular collapse, segmental necrosis and finally glomerulosclerosis. The resulting glomerular filtration barrier damage causes proteinuria (Klahr, 1988; Mennuni *et al*., 2013). In addition there is a fall in renal blood flow that correlates directly with the degree of renal vascular damage and the severity and duration of hypertension, and inversely with the BP level (De Leeuw and Birkenhager, 1983). The end result is progressive fibrosis and scarring that causes glomerular and tubulointerstitial damage, leading to nephrosclerosis, renal insufficiency and loss of renal function `(Haraldson *et al*., 2008; Shankland, 2006).

Assessment of renal damage due to hypertension is based on the diagnosis and progression of CKD which is categorized according to 5 stages with stage 5 being the most severe i.e. ESRF (Table 1.4). Glomerular renal damage is indicated by the presence of proteinuria or more specifically microalbuminuria. Proteinuria levels of more than 300 mg/day is a hallmark of renal damage, whereas values between 30 and 300 mg/day is considered a predictor of future renal damage (Elliot, 2013). At present, urine albumin level is commonly used as a biomarker of glomerular renal damage. In normal kidney function, very little albumin is excreted by the kidney. However in hypertensive renal injury, glomerular filtration of albumin is increased due to structural and functional transformation processes in the glomeruli that causes increased permeability (Schmeider, 2010). The rate of albumin excretion has been found to correlate with BP levels (Parving *et al*., 1974). Albuminuria is classified according to range of severity i.e. microalbuminuria (30-300 mg/day), macroalbuminuria (300 mg-3 g/day) and nephritic range albuminuria (> 3 g/day) (Tesch, 2010). Studies have indicated that increased microalbuminuria levels are associated with subclinical glomerular renal damage (Pontremoli *et al*., 2002).

Besides absolute urine protein or albumin values, proteinuria and microalbuminuria are also expressed as a ratio to urine creatinine values. Renal damage is indicated by a urine protein/creatinine value greater than 45 mg/mmol or an albumin/creatinine value exceeding 30 mg/mmol. Reduced glomerular filtration rate (GFR), whether measured or estimated by calculation (eGFR) is another measure of glomerular damage. A GFR of less than 60 ml/minute defines the nominal boundary of clinically significant CKD (Stevens and Levey, 2005). Renal tubulointerstitial damage due to hypertension can be detected by urine N-acetyl-beta-D-glucosaminidase (NAG) levels. NAG is a proximal tubular lysosomal enzyme that is released during damage to proximal tubules (Bazzi *et al*., 2002). Increased levels of

urine NAG have been reported in untreated essential hypertension and have been recommended as a screening test for renal damage (Mansell *et al*., 1978; Maruhn, 1976; Alderman *et al*., 1983).

1.2.1.4 Management of hypertension

The main goal of treating essential hypertension is not only to reduce BP to normal levels but to also prevent the complications associated with elevated BP, extend longevity and improve the quality of life. Lowering of blood pressure is always preferable by non-pharmacological means that do not involve antihypertensive drugs (Messerli *et al*., 2007). As such, initially lifestyle modifications might be attempted for prehypertension, borderline or mild hypertension. This includes diet changes involving reduced sodium intake, increasing intake of whole grains, fruits and vegetables and reducing or avoiding alcohol consumption (Sachs and Campos, 2010). Other lifestyle modifications include reducing body weight and increased physical activity (Crawford, 2003; Savica *et al*.,2010). However if lifestyle modifications alone are not successful, pharmacologic therapy in the form of antihypertensive drugs have to be instituted as well so as to obtain an optimal BP level which traditionally has been targeted as less than 140/90 mm Hg (Ram, 2014). Depending on the condition of the patient, antihypertensive drug treatment can be commenced as monotherapy involving a single antihypertensive drug or if unsuccessful, as combination therapy involving 2 or more drugs (Chobanian, 2009).

Antihypertensive drugs are classified according to their site or mode of action. At present the commonly used classes of antihypertensive drugs are diuretics, angiotensin converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARB), beta blockers and calcium channel blockers (CCB). Less commonly used classes of antihypertensive drugs include the central adrenergic inhibitors and alpha

blockers. The major classes of antihypertensive drugs, their mechanism of action and clinical uses are as shown in Table 1.4

1.2.1.5 Problems and limitations in current management

There are a number of problems and limitations in the current management of essential hypertension using antihypertensive drugs. First and foremost is the side-effects and adverse effects that these drugs cause. All the different classes of drugs have some side / adverse effects, ranging from mild to severe and even life threatening, that affect the wellbeing, quality of life and health of the patients (Beevers *et al*., 2001; Cohuet and Struijker-Boudier 2005; Kaur and Khannab, 2012). This problem becomes worse when combination therapy involving 2-3 different classes of drugs is required, which magnifies the side / adverse effects that patients have to face. Studies have shown that more than two-thirds of hypertensive individuals need combination therapy for adequate control of BP (Cushman *et al*., 2002; Dahlof *et al*., 2002).