# EFFECT OF EXOGENOUSLY ADMINISTERED HYDROGEN SULPHIDE ($H_2S$) ON PLASMA CONCENTRATION OF NUCLEAR FACTOR-KAPPA B (NF-kB) AND INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) IN A RAT MODEL OF RENAL ISCHEMIA REPERFUSION INJURY

**by**

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| | |
|---|---|
| IRI | Ischemia reperfusion injury |
| I/R | Ischemia reperfusion |
| ROS | Reactive oxygen species |
| ARF | Acute renal failure |
| Cisp | Cisplatin |
| HTN | Hypertension |
| NIBP | Non-invasive blood pressure |
| SBP | Systolic blood pressure |
| DBP | Diastolic blood pressure |
| MAP | Mean arterial pressure |
| HR | Heart rate |
| PP | Pulse pressure |
| RCBP | Renal cortical blood perfusion |
| PWV | Pulse wave velocity |
| L-NAME | L-nitro arginine methyl ester |
| NO | Nitric oxide |
| NOS | Nitric oxide synthase |
| eNOS | Endothelial nitric oxide synthase |
| nNOS | Neuronal nitric oxide synthase |
| $O_2^{\bullet -}$ | Superoxide anion |
| $H_2O_2$ | hydrogen peroxide |
| $ONOO^-$ | Peroxynitrite |
| $OH^-$ | Hydroxyl radical |

| | |
|---|---|
| ICAM-1 | Inter cellular adhesion molecule-1 |
| CD 54 | Cluster of differentiation |
| Ig | Immunoglobulin |
| IL-1 | Inter leukin-1 |
| TNF-α | Tumor necrosis factor-α |
| LFA-1 | Lymphocyte function associated antigen-1 |
| NF-kB | Nuclear factor kappa-B |
| DNA | Deoxyribonucleic acid |
| mRNA | Messenger ribonucleic acid |
| IkB | Inhibitory kappa B |
| $H_2S$ | Hydrogen sulphide |
| CBS | Cystathionine beta synthase |
| CSE | Cystathionine gamma lyase |
| 3-MST | 3-Mercaptopyruvate sulfurtransferase |
| PAG | dL-propargylglycine |
| NaHS | Sodium hydrosulphide |
| WKY | Wistar Kyoto |
| ATN | Acute tubular necrosis |
| MDA | Malondialdehyde |
| T-SOD | Total superoxide dismutase |
| GSH | Glutathione |
| G | Grams |
| mmHg | Millimetre mercury |
| mg/mL | Milligram per milliliter |
| µmol/kg | Micromole per kilogram |

| | |
|---|---|
| mM | Millimole |
| nmol/mL | Nanomole per milliliter |
| pg/mL | Picogram per milliliter |
| Ng | Nanogram |
| mL/min/kg | Millilitre per minute per kilogram |
| mg/kg | Milligram per kilogram |
| O.D | Optical density |
| HRP | Horse radish peroxidase |
| i.p. | Intraperitoneal |
| mL | Millilitre |
| % | Percentage |
| m/s | Meter per second |
| µM | Micro mole |
| µL | Microliter |
| Ucr. | Urinary creatinine |
| Pcr. | Plasma creatinine |
| BW | Body weight |
| $U_{Na}V$ | Absolute urinary sodium excretion |
| $U_kV$ | Absolute urinary potassium excretion |
| $FE_{Na+}$ | Fractional excretion of sodium |
| $FE_{K+}$ | Fractional excretion of potassium |
| Na:K ratio | Sodium : potassium ratio |
| D.W | Distal water |
| W/V | Weight/ per volume |
| KI | Kidney index |

| | |
|---|---|
| UFR | Urine flow rate |
| BPU | Blood perfusion unit |
| PP tubing | Polypropylene tubing |
| Abs. | Absorbance |
| Conc. | Concentration |
| Std | Standard |

**KESAN PEMBERIAN HIDROGEN SULFIDA ($H_2S$) KE ATAS KEPEKATAN NUKLEAR FAKTOR KAPPA-B (NF-KB) DALAM PLASMA DAN MOLECUL-1 LEKATAN INTERSEL (ICAM-1) DI DALAM MODEL TIKUS YANG MENGALAMI KECEDERAAN REPERFUSI ISKEMIA GINJAL**

## ABSTRAK

Kajian ini menyelidik kesan pemberian penderma hidrogen sulfida ($H_2S$), sodium hidrosulphida (NaHS) secara eksogenous ke atas kepekatan nuklear faktor kappa-B (NF-kB) dan molekul-1 intersel (ICAM-1) dalam kecederaan iskemia reperfusi ginjal di dalam tikus normotensif *Wistar Kyoto* (WKY) tanpa kegagalan ginjal (NRF) dan dengan kegagalan ginjal (RF) serta hipertensi aruhan L-nitro-arginin-methil-ester (L-NAME) di dalam tikus tanpa kegagalan ginjal (NRF) dan dengan kegagalan ginjal (NRF). NaHS telah disuntik secara intraperitoneal pada dos 56 µmol/kg setiap hari selama 35 hari. Hipertensi telah diaruh di dalam tikus WKY dengan memulakan pemberian L-NAME secara oral dalam air minuman pada hari ke 8 dalam protokol kajian ini pada dos 40mg/kg selama 28 hari. Penyakit kegagalan ginjal akut (ARF) telah diaruh dengan suntikan tunggal cisplatin secara intraperitoneal pada dos 5 mg/kg. Penghalang cistationin gama liase (CSE) dL-propargilglicin (PAG) telah diberi pada hari ke-36 (hari eksperimen akut) secara suntikan tunggal intraperitoneal pada dos 50mg/kg. Haiwan telah dibahagikan kepada 4 kumpulan utama (i)WKY, (ii) L-NAME, (iii) WKY-RF dan (iv) L-NAME-RF. Semua kumpulan-kumpulan tersebut telah dipecahbahagikan kepada tiga kumpulan kecil lagi iaitu (i) kumpulan kawalan, (ii) kumpulan rawatan NaHS dan (iii) kumpulan yang diberi PAG. Eksperimen kawalan masa juga telah dilakukan dengan menjalankan eksperimen sham di dalam kumpulan-kumpulan WKY-SHAM dan L-NAME-SHAM dimana iskemia tidak diaruh. Parameter-parameter data

fisiologi, hemodinamik sistemik dan fungsi ginjal telah diambil pada hari ke-0, 21 dan 35 di samping pengukuran $H_2S$ di dalam plasma pada hari ke-0, 21 dan 36 kajian. Hemodinamik sistemik, tekanan darah kortikal ginjal (RCBP), halaju gelombang denyutan (PWV), parameter fungsi ginjal, penanda tekanan oksidatif, kepekatan ICAM-1 dan NF-kB juga telah diukur pada hari akut eksperimen (hari ke-36) di fasa pra-iskemia dan fasa reperfusi. Data diberikan sebagai min ± S.E.M dan telah dianalisis dengan menggunakan langkah-langkah berulang analisis varians (ANOVA) satu-hala dan diikuti dengan ujian pos hoc dengan singnifikasi $P<0.05$. Pra-rawatan dengan NaHS telah membaikpulih parameter fungsi ginjal di dalam kumpulan ARF dan menurunkan hipertensi aruhan L-NAME secara signifikan (semua adalah $P<0.05$). Tambahan pula, rawatan dengan NaHS telah membaikpulihkan paras kecederaan reperfusi iskemia ginjal (IRI) yang dibuktikan dengan pengurangan signifikan oleh penanda tekanan oksidatif (semua adalah $P<0.05$), indeks ginjal dan peningkatan signifikan di dalam RCBP (semua adalah $P<0.05$) yang diikuti IRI ginjal. Pra-rawatan dengan NaHS juga telah menurunkan kepekatan ICAM-1 dan NF-kB secara signifikan (semua adalah $P<0.05$) selepas IRI ginjal. Kesimpulannya, $H_2S$ mempunyai potensi anti oksidan seperti yang terbukti dalam kajian ini dengan menurunkan intensiti tekanan oksidatif yang dimana sebagai tindak balas telah menghalang pengaktifan NF-kB. Selain itu, $H_2S$ juga mempunyai ciri-ciri anti radang yang dibuktikan di dalam kajian ini melalui penurunan kepekatan ICAM-1. Oleh itu, penemuan ini menunjukkan bahawa $H_2S$ mempunyai kedua-dua anti-oksida dan anti-radang yang menyumbang kepada pengurangan dalam keterukan kerosakan IRI buah pinggang.

**EFFECT OF EXOGENOUSLY ADMINISTERED HYDROGEN SULPHIDE ($H_2S$) ON PLASMA CONCENTRATION OF NUCLEAR FACTOR-KAPPA B (NF-kB) AND INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) IN A RAT MODEL OF RENAL ISCHEMIA REPERFUSION INJURY**

## ABSTRACT

The present study investigated the effect of exogenous administration of hydrogen sulphide ($H_2S$) donor, sodium hydrosulphide (NaHS) on the concentration of nuclear factor kappa-B (NF-kB) and inter cellular adhesion molecule-1 (ICAM-1) in renal ischemia reperfusion injury in normotensive *Wistar Kyoto* (WKY) non-renal failure (NRF) and renal failure (RF) rats as well as L-nitro-arginine-methyl-ester (L-NAME) induced hypertensive NRF and RF rats. NaHS was administered intraperitoneally at a dose of 56 µmol/kg on daily basis for 35 days. Hypertension was induced in WKY rats by starting oral administration of L-NAME in drinking water on day 8 of the study protocol at a dose of 40 mg/kg for 28 days. ARF was induced by single intraperitoneal injection of cisplatin at a dose of 5 mg/kg. Cystathionine gamma lyase (CSE) inhibitor dL-propargylglycine (PAG) was administered on day 36 (acute experiment day) by single intraperitoneal injection at a dose of 50 mg/kg. All the animals were divided into four main groups (i) WKY, (ii) L-NAME, (iii) WKY-RF and (iv) L-NAME-RF. All these groups were further sub-divided into three sub groups (i) control groups, (ii) NaHS treatment groups and (iii) PAG administered groups. Time control experiments were also performed by operating sham experiments in WKY-SHAM and L-NAME-SHAM groups in which ischemia was not induced. Physiological data, systemic haemodynamics and renal functional parameters were measured on days 0, 21 and 35 along with plasma $H_2S$

measurement on days 0, 21 and 36 of the study protocol. Similarly systemic haemodynamics, RCBP, PWV, renal functional parameters, oxidative stress markers, ICAM-1 and NF-kB concentration were also measured on acute experiment day (day 36) at pre-ischemic phase and reperfusion phase. Data, mean ± SEM were subjected to repeated measure one-way ANOVA followed by Bonferroni *post hoc* test with significance at $P<0.05$. Pre-treatment with NaHS improved renal functional parameters in ARF groups as well as decreased significantly (all $P<0.05$) the L-NAME induced hypertension. Moreover, pre-treatment with NaHS reduced the severity of renal ischemia reperfusion injury (IRI) as evidenced by significant reduction (all $P<0.05$) of oxidative stress markers, kidney index and significant increase (all $P<0.05$) in RCBP following renal IRI. Similarly, pre-treatment with NaHS also decreased significantly (all $P<0.05$) NF-kB and ICAM-1 concentration after renal IRI. In conclusion, $H_2S$ has anti-oxidant potential as evidenced in the present study by reducing the intensity of oxidative stress which in response inhibited the activation of NF-kB. Similarly, $H_2S$ has also anti-inflammatory properties as evidenced in the present study by the down regulation of ICAM-1 concentration. Hence, these findings indicate that $H_2S$ has both anti-oxidant and anti-inflammatory properties which contribute to a reduction in severity of renal IRI damage.

# CHAPTER 1

# INTRODUCTION

## 1.1 Kidney

### 1.1.1 Anatomy of the kidney

Human beings have a pair of kidneys which are present on each side of the spine in the abdominal cavity. The left kidney is located at the vertebral level T12-L3 in the retroperitoneal space while, the right kidney is slightly lower than the left kidney. The position of the left kidney is approximately beneath the diaphragm posterior to the spleen. The right kidney is located beneath the diaphragm and posterior to the liver. An adrenal gland is present on the top of each kidney. Upper parts of the kidneys are partially protected by the 11th and 12th ribs. Both kidneys along with their adrenal glands are surrounded by two layers of fats the perirenal and the pararenal fat and the renal fascia (Walter & Boron, 2004). The weight of the adult male kidney ranges from 125 gm to 170 gm and 115gm to 155 gm in adult females. The kidney is approximately 11-14 cm long, 6 cm wide and 4 cm thick (Glodny et al., 2009). Kidney is a bean shaped structure consisting of concave and convex borders. The depression on the concave surface of the kidney is called the renal hilum. At the renal hilum the renal artery and renal vein enters into the kidney while, renal vein and the ureter leaves the kidney at this position. (Marieb & Hoehn, 2007). The cross section of the kidney shows two regions, the outer reddish brown

part called cortex and the inner darker brown part is called medulla. Nephron is the structural and functional unit of the kidney and extends across the cortex and the medulla (Shier, 2003). Each kidney consists of approximately 20 million nephrons. Nephron consists of glomerulus and the bowmans capsule and this portion is called the renal corpuscle and is located in the cortex. Renal corpuscle is then followed by the renal tubules which passes into medullary pyramids (Walter & Boron, 2004). The renal tubules consist of the proximal convoluted tubules, loop of henle and distal convoluted tubules. Tip of each pyramid empties the urine into the minor calyx which further empties urine into the major calyces and finally the major calyces empties urine into renal pelvis and then the ureter (Drake et al., 2005). Right and left renal artery supplies blood directly from the abdominal aorta into the respective kidneys. Despite small size of the kidneys they still receive approximately 20 % of the cardiac output. Each of the renal arteries divides into segmental arteries which branch further into the interlobar arteries which enter into the renal capsule and further extend into the renal columns. The arcuate arteries receive blood from the interlobar arteries that extend across the surface of the medulla and cortex. Each of the arcuate artery empties into various interlobular arteries which further extend into the afferent arterioles and these arterioles supplies blood into the glomeruli (Walter & Boron, 2004). Once the blood is filtered it passes through small venules followed by interlobular veins. From these veins blood is supplied to arcuate veins and then to interlobar veins which finally empty into the renal vein. Blood from both renal veins returns into inferior vena cava. (Applegate, 2000; Meyer et al., 2004; Vander, 1995).

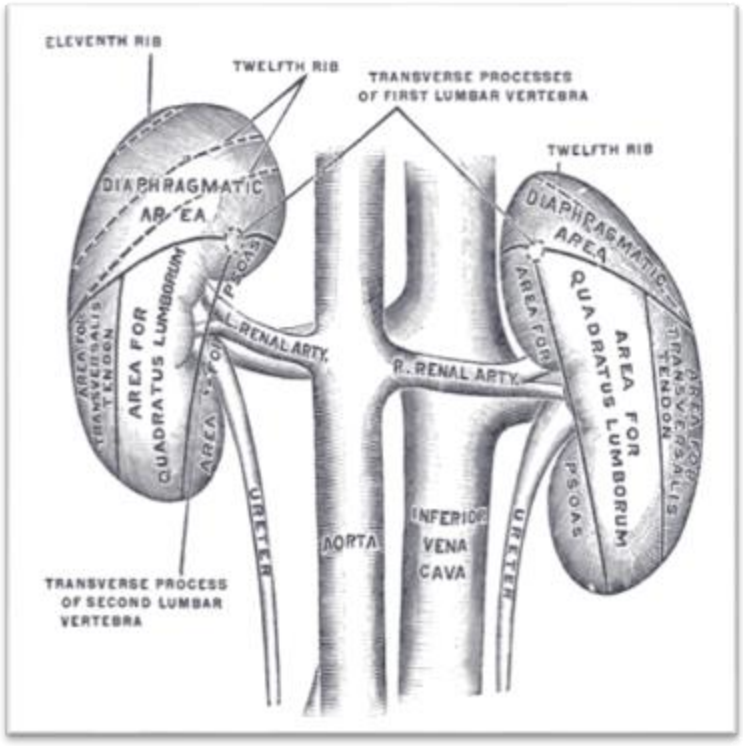


**Figure 1.1:** Anatomy of the human kidneys. This picture has been adopted from Gray, Henry. 1918. Anatomy of the human body (Gray, 1918).

### 1.1.2 Renal physiology

The function of the kidneys is to excrete a wide range of waste products produced by the process of metabolism like urea, nucleic acid and uric acid. Kidneys are also responsible for the reabsorption of many vital nutrients like glucose and amino acids. Kidneys play a key role in the acid base homeostasis and hormones like erythropoietin is also produced by the kidneys (Dantzler, 1989). Kidneys play a vital role in the regulation of blood pressure through the renin angiotensin aldosterone system (Hall & Guyton, 2011). Kidneys contribute in maintaining blood pressure along with nervous, cardiovascular and endocrine systems (Germann et al., 2005). Nephron is responsible for the process of filtration followed by reabsorption and finally secretion. The process of filtration takes place at the renal corpuscle. From

the blood larger proteins and various cells get filtered in the process of filtration. Thus, the glomerulus filtrate is produced which finally forms the urine. The production of the ultra-filtrate is approximately 180 liters per day of which a large portion is reabsorbed by the kidneys. Thus the production of urine is only about 2 liters a day (Guyton, 1991).

## 1.2 Ischemia reperfusion injury

### 1.2.1 Ischemia

It is a condition in which blood supply to an organ or tissue is reduced or restricted. This results in deficiency of oxygen and glucose supply which is necessary for the process of cellular metabolism (Siemionow & Arslan, 2004). Ischemic injury is the stoppage or interruption in the arterial blood supply with the sudden oxygen shortage to the cells. Thus hypoxia is produced along with augmentation of metabolic products (Kosieradzki & Rowinski, 2008).

There are different types of ischemia like (a) myocardial ischemia, (b) cerebral ischemia, (c) critical limb ischemia, (d) hepatic ischemia and (e) renal ischemia.

### 1.2.1 (a) Myocardial ischemia

It is a condition in which the heart is unable to maintain the balance between blood supply and the demand of oxygenated blood to myocardium itself due to blockade or narrowing of the coronary arteries (Mohan, 2010). Myocardial ischemia is also called ischemic heart disease (IHD).

### 1.2.1 (b) Cerebral ischemia

A condition in which the blood supply to the brain is not sufficient to meet the needs for the metabolic processes (Ames et al., 1968). Cerebral ischemia is also called brain or cerebrovascular ischemia.

### 1.2.1 (c) Critical limb ischemia

It is the condition in which there is insufficient blood supply to the hands, legs and feet due to severe blockade of the arteries. As a result there is pain at rest in the form of continuous burning sensation of the legs or feet (Schanzer & Conte, 2010). There are also sometimes ischemic skin ulcers and even gangrene (Minar, 2009).

### 1.2.1 (d) Hepatic ischemia

It is the condition in which there is interruption of blood supply to the liver as a result the liver does not receive enough blood supply or oxygen (Lentsch et al., 2000).

### 1.2.1 (e) Renal ischemia

Renal ischemia is the condition in which there is complete cut off or reduced blood supply thus, a hypoxic state is developed in the kidneys and due to this insufficent oxygen the injury is initiated by the breakdown of ATP. As a result of this, adenosine and other metabolic products of nucleotides move out of the cells causing ATP production to be delayed once the blood is reperfused back (Gaudio et

al., 1986). Functional and structural integrity of all cells is dependent upon cellular tight junctions and microfilaments and its functioning requires a continuous supply of energy. Once the energy supply is stopped protein subunits breakdown and they leave the tight junction of the cells and cellular microfilaments (Hays, 1992). There is evidence that the concentration of free intercellular ions increases during ischemia (Lee & Allen, 1992) and the injury to kidneys caused by the ischemic condition is the main reason for acute renal failure (Basile et al., 2003). Various organ transplantation failures are due to this ischemic damage (Wolfs et al., 2005). Some of the effects of ischemia at the cellular level are: altered membrane potential, changes in ion distribution, cytoskeletal disorganization, cellular swelling, cellular acidosis, increased hypoxanthine production and decreased phosphocreatinine, glutathione and ATP production (Collard & Gelman, 2001).

### 1.2.2 Reperfusion injury

Reperfusion injury is the damage induced by the reperfused blood as it is restored after the ischemic phase. Reperfusion injury is the result of cell activation and the injury occurred during ischemia (Bilzer et al., 1999). In reperfusion phase the permeability of various capillaries and arterioles becomes much more high and thus the tissues becomes more susceptible to fluid filtration. After the reperfusion process the concentration of reactive oxygen species is increased because they are produced in greater quantity by the activated endothelial cells but the nitric oxide production is reduced. This imbalance between the reactive oxygen species and nitric oxide production initiates the inflammatory response (Carden & Granger, 2000). Reperfusion injury is the effective continuation stage of the injury induced by ischemia. This injury may occur within hours or days after ischemia. Cellular

necrosis and apoptosis is accompanied together by the cellular regeneration and repairing processes which requiring energy. The activation of caspase and liberation of cytochrome C occurs as early as 5 minutes after the process of reperfusion (Hoek et al., 2003). The concentration of free intercellular calcium ion which was increased during early ischemia starts returning back to the normal level. Due to this the cells start recovering from early damage done in ischemic phase. But due to continuous increase of the calcium ion an irreversible injury starts (Lee & Allen, 1992). After reperfusion there is a quick release of free radicals. The main source for the production of free radicals is respiratory chain enzymes. Superoxide is formed from free oxygen which reacts with reduced ubiquinone. Superoxide is metabolized by catalyse to produce hydrogen peroxide or this superoxide is metabolized to hydroxyl radical where ferrous or copper acts as catalyst (Kosieradzki & Rowinski, 2008). After reperfusion, the superoxide free radical $\cdot O^-_2$ is produced in large quantities. This reactive superoxide specie produces more hydroxyl radical $\cdot OH$, through Fenton and Haber-Weiss reactions and uses iron as a reaction catalyst (Zweier, 1988).

$$O^-_2 + Fe^{3+} \longrightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH$$

These highly reactive free radicals cause the peroxidation of the mitochondrial and cellular membranes of the phospholipids and hence induce endothelial injury in reperfusion phase. This causes an increase in the extent of injury of endoplasmic reticulum which causes the production of autophagosomes and then cellular degradation. This increases lysosomal activity of the proteases and causes further necrosis and aptoposis (Kosieradzki & Rowinski, 2008). Metabolites of reactive oxygen species and polymorphonuclear leukocytes have been reported to

be involved in the pathophysiology of ischemia reperfusion injury (Zimmerman & Granger, 1994). The reactive oxygen species produced during ischemia reperfusion injury is responsible for the cellular damage through lipid peroxidation (Jaeschke, 1998). It has also been cited that one of the most important hypothesis of cellular damage by the reactive oxygen species is through inhibition of lipid peroxidation (Jaeschke, 2000). Cellular damage caused by the reactive oxygen species is due to the degradation and oxidation of proteins, lipid peroxidation and also due to the damage of DNA. These redox signalling injuries activates the pathways of transduction which in response initiates the process of injury (Fan et al., 1999). It has also been hypothesized that xanthine oxidase and mitochondria is a potential source of reactive oxygen species formation (Jaeschke, 1996). One study has suggested that generation of reactive oxygen species by phagocytic cells may be one source for the cellular injury but different types of inflammatory mediators may also be involved in the reperfusion injury (Jaeschke et al., 1992). These reactive oxygen species are responsible for the cellular damage and also act as second messenger for the initiation of inflammation. The major site for the production of reactive oxygen species is mitochondria. The consumption of oxygen and production of energy is achieved by the electron transport chain. In this transport chain the molecular oxygen is reduced to water. The ischemic reperfusion injury in mitochondria is responsible for the electron transport chain decoupling. As a result of this decoupling large quantity of reactive oxygen species is generated and released (Fan et al., 1999). So, reactive oxygen species is an important factor during ischemia reperfusion injury. Ischemic reperfusion injury is also a major factor for the increased morbidity and mortality in numerous clinical conditions specially in organ transplantation failure (Fan et al., 1999).

### 1.3 Renal failure

Renal failure is characterized by renal dysfunction in which kidneys are unable to perform filtration of waste products in the blood properly (Kajbaf et al., 2013). There are two major types of renal failure discussed below.

#### 1.3.1 Chronic renal failure

Chronic renal failure is a condition which is characterized by the kidney injury and glomerular filtration rate becomes lower than 60 mL/min for a period of three consecutive months or a period of more than three months. In chronic renal failure an irreversible sequence of damages occur which causes last stage kidney disease (Parmar, 2002). Chronic kidney injury involves the irreversible loss of kidney function involving slow deterioration of parenchymal cells of the kidney leading to nephronal damage during this process (Mohan, 2010).

#### 1.3.2 Acute renal failure

In acute renal failure (ARF), renal function is affected or declined in a shorter span of time between hours to days and may result in kidney failure. The kidney is unable to perform excretion of various waste products as well as balance and maintenance of electrolytes and fluids (Ympa et al., 2005). Acute renal failure involves rapid onset of renal dysfucntion mainly indicated by anuria or oliguria. ARF is also characterized by rapid increase of blood creatinine and urea concentration and ureamia may also occur subsequently (Mohan, 2010). In acute renal failure the concentration of creatinine in serum increases 50 % from the

baseline value (Evenepoel, 2004). Various factors which induce ARF are hypovolemia, sepsis, liver dysfunction, damaged renal perfusion, vascular blockade and drugs (Ympa et al., 2005). Three main causes of ARF has been identified (Mohan, 2010) which are discussed below.

### 1.3.2 (a) Pre-renal cause

It is a reversible type of renal failure and reduced arterial blood flow to the kidneys is the main reason for pre-renal azotaemia (Evenepoel, 2004). It is characterized by reversible increase of plasma creatinine and blood urea. Decreased renal reperfusion is the cause of pre-renal azotaemia due to which the glomerular filtration rate becomes low (Lameire, 2005). It occurs in response to low extracellular volume and increased activity of adrenergic receptors and may accompany heart failure, sepsis, cirrhosis or nephrosis (Blantz, 1998).

### 1.3.2 (b) Intra-renal cause

In intra renal acute renal failure damage to the renal tissues occurs specially to the renal arteries and arterioles. Moreover, it is characterised by injury to glomeruli and acute tubular necrosis (ATN) (Mohan, 2010). Various toxins and ischemia is mainly responsible for the tubular injury occurring in intra-renal ARF (Wali & Henrich, 2002).

### 1.3.2 (c) Post-renal cause

Post-renal cause involves blockade to urine flow in the renal tract. This obstruction to the flow of urine results from the formation of a mass inside the lumen or there may be any external compression on the urinary tract, urethra, ureter or bladder which leads to renal infection (Mohan, 2010).

### 1.3.3 Drugs-induced acute renal failure

ARF may be induced by different types of therapeutic agents. Acute interstitial nephritis results from various drugs which causes allergic reactions. This may further cause inflammation and tubular injury. Therapeutic agents that cause ATN involve toxicity to the tubular epithelium directly with the induction of inflammation. Some other injuries induced by various medications involves crystal nephropathy, acute nephrocalcinosis and osmotic nephropathy (Markowitz & Perazella, 2005). Therapeutic agents that cause pre-renal acute renal failure include NSAIDS, cyclosporine and diuretics etc (Lameire, 2006). Drugs responsible for the post-renal causes of acute renal failure include methysergide, acyclovir, sulphonamide and triamterene etc (Perazella, 1999). Majority of drugs and chemicals are involved in the induction of intra-renal ARF (Woolfson & Hillman, 2000; Zager, 1997). Amongst various tubular injuries ATN is the most prominet one that occur during intra-renal injury of acute renal failure (Woolfson & Hillman, 2000). Various chemical agents and drugs that cause ATN are antibiotics, amphoteracin B, aminoglycosides, cyclosporine A, mannitol, cisplatin and polyethylene glycol etc

### 1.3.3 (a) Cisplatin induced acute renal failure

Cisplatin belongs to the class of platinum containing anti-cancer drugs used for solid malignancy treatment. Cisplatin is also used in the treatment of lung cancer, bladder cancer, ovarian cancer, cervical cancer, germ cell tumours, lymphomas and sarcomas. Various models of ARF in laboratory animals for different physiological and pharmacological studies have been achieved through cisplatin administration (Atessahin et al., 2005; Rubera et al., 2013; Tayem et al., 2006). Tubular filtration and secretion are mainly responsible for the excretion of cisplatin whereas peritubular uptake is responsible for cisplatin accumulation in the kidneys (Annie et al., 2005). So, cisplatin does not undergo the process of tubular reabsorption. Proximal tubular cells of outer medulla and inner cortex are the main site for cisplatin toxicity. Mostly cisplatin-induced renal injury occurs at S3 segment of nephrons (Leibbrandt et al., 1995). It has been reported by various studies that renal toxicity occurs by injecting single dose of cisplatin. Renal toxicity causes an increase in serum creatinine and blood urea nitrogen. Cisplatin renal toxicity induces necrosis and apoptosis of proximal convoluted tubules (Dobyan et al., 1980). Cisplatin induced renal toxicity causes ATN and sloughing of epithelial cells of the renal tubules (Al-Harbi et al., 1995), decreased glomerular filtration rate (Kang et al., 2004), damage to proximal tubules (Jones et al., 1985) and increased resistance to renal blood vessels (Matsushima et al., 1998). Cisplatin induce renal failure also involves the role of reactive oxygen species (ROS) specially hydroxyl radicals and superoxide anion because it has been reported that oxidative stress is induced by cisplatin due to the production of free radicals (Badary et al., 2005; Brahmi et al., 2012). Increased peroxidation of lipid molecules is also a hallmark of cisplatin induced renal toxicty (Paul et al., 1991; Vaziri et al., 2002). It has also been reported

that ROS involves in the inhibition of various anti-oxidant enzymes in the kidneys (Masuda et al., 1994) and specially affecting the concentration of renal glutathione level (Jin & Lindup, 1993).

### 1.4 Hypertension

Chronic increase of systemic arterial blood pressure above defined limits is defined as hypertension. Thus, hypertension is a chronic medical condition sometimes also called as high blood pressure or arterial hypertension. The border line between normal pressure and hypertension is 140/90 mmHg (Vander & Luciano). So, hypertension is sustained increase of systolic blood pressure which exceeds 140 mmHg and diastolic blood pressure exceeds 90 mmHg in adults. Hypertension is a common cause of various cardiovascular diseases like coronary artery disease, cardiac failure and dissecting aortic aneurysm. It is also a risk factor for renal insufficiency and stroke (Laurence et al., 2008).

**Table 1.1:** Classification of hypertension for adults in Malaysia (NHMS 3, 2006)[3] redrawn by using Ms Word office from Clinical Practice Guidelines for Management of Hypertension (4th edition) issued in 2013 available online on http://www.moh.gov.my/attachments/CPG_Management_of_Hypertension_4th_Edition.pdf

| **Classification** | **Systolic B.P (mm Hg)** | **Diastolic B.P (mm Hg)** |
|---|---|---|
| Optimal | < 120 and | < 80 |
| Normal | < 130 and | < 85 |
| High normal | 130 – 139 and/or | 85 – 89 |
| **Hypertension** | | |
| Stage 1 | 140 – 159 and/or | 90 – 99 |
| Stage II | 160 – 179 and/or | 100 – 109 |
| Stage III | ≥ 180 and/or | ≥ 110 |

### 1.4.1 Primary hypertension

It is the type of hypertension for which the cause or origin is unknown. It is also called essential hypertension. Most of the patients having essential hypertension have a family history indicating hereditary tendency (Guyton & Hall, 2006). It has been citied in various articles that genetics is a key factor in the primary hypertension (Feinleib et al., 1977; Longini et al., 1984). Demographical and environmental causes are the factors mainly responsible for genetically induced hypertension (Oparil et al., 2003). Obesity may also be the cause for the primary hypertension (Chiong et al., 2008).

### 1.4.2 Secondary hypertension

It is the type of hypertension for which the cause or origin is known (Chiong et al., 2008; Guyton & Hall, 2006). About 5-10 % of the population suffer from secondary hypertension. The major causes of secondary hypertension are various diseases some of them are given as follow.

Renal diseases like renal parenchymal disease, renin producing tumor and renal vascular disease etc. Cardiovascular diseases like rigidity of aorta and coarctation of aorta. Endocrine diseases like hypothyroidism, hyperthyroidism, primary aldosteronism and acromegaly etc. Acute stress related hypertension, pregnancy related hypertension, obstructive sleep apnea and drugs induced hypertension (Chiong et al., 2008).

### 1.4.2 (a) L-NAME induced hypertension

L-nitro-arginine methyl ester (L-NAME) induced hypertension is widely used as an animal model for secondary hypertension in various *in vivo* studies. L-NAME given orally to the laboratory animals induces hypertension and thus presents as a secondary hypertension model in laboratory rats (Khayyal et al., 2002). L-NAME is a nitric oxide synthase (NOS) inhibitor and chronic treatment with L-NAME induces arterial hypertension in rats. Systemic blood pressure and vascular tone is mainly regulated by the endothelium which is involved in the production of various vasodilating and vasoconstricing factors like nitric oxide (NO), angiotensin II, endothelins and prostacyclin (Bernatova et al., 2000). Various studies have shown that L-arginine is the precursor of NO which mediates various gastrointestinal, neural and cardiovascular functions. L-arginine is metabolised to NO by NOS

(Mourad et al., 1996). NO in the blood vessels is mainly produced from the isoforms of NOS either by endothelial nitric oxide synthase (eNOS) or nitric oxide synthase-3 (NOS3). More studies have also reported that NO is produced endogenously and its continuous genesis plays a key role in the maintenance of vascular and gastric integrity (Tepperman & Whittle, 1992; Whittle et al., 1990). Inhibition of platelet aggregation, inhibition of neutrophil endothelium adhesion, regulation of apoptosis, and vasodilation is mainly induced by NO (Rosselli et al., 1998). L-NAME inhibits eNOS because it is an analogue of the L-arginine and thus competes with it on eNOS binding sites (Rees et al., 1990). L-NAME causes the inhibition of eNOS thus inactivating NO. It also inhibits nNOS and thus stimulates sympathetic nervous system. In order to maintain hypertension L-NAME activates renin-angiotensin system (Biancardi et al., 2007). Moreover, various mechanisms of L-NAME induced hypertension have also been reported like, L-NAME causes an increase in the level of endothelin-1 (Amours et al., 1999) and also blocks $Na^{+}K^{+}$-ATPase channels (Rossoni et al., 2003) and thus contributes to the induction of hypertension. It is also documented that L-NAME may act as an antagonist for the muscarinic receptors and may also effect the activity of prostaglandins (Buxton et al., 1993). Long term treatment with L-NAME *in vivo* studies show structural changes in the vasculature and may also cause myocardial hypertrophy (Usui et al., 1999). Decrease in the endogenous production of NO may cause oxidative stress (Kurose et al., 1995; Niu et al., 1994). Increased oxidative stress in the rats treated with L-NAME is reported further evidenced by increased protein oxidation of the membrane, enhanced NADPH oxidase-1 (NOX-1) and NADPH oxidase-2 (NOX-2) and also augmented concentration of superoxide dismutase (SOD) which is an anti-oxidant glutathione (Bell et al., 2008). Inhibition of NO by L-NAME may cause the production of

reactive oxygen species (ROS) specially superoxide anion ($O^{-}_{2}$) (Kitamoto et al., 2000).

### 1.5 Inflammation

Inflammation is the local response of the tissues to any injury induced by any harmful agent or stimuli and is therefore a defensive mechanism of the body which involves the removal of the harmful agents or reducing the chances of spreading of these injurious agents (Mohan, 2010). It is the process in which body response to various stimuli like irritants, various pathogens and some damaged cells (Ferrero et al., 2007). During inflammation there is release of different types of mediators by injured or damaged cells in response to heat, trauma or different types of chemicals. This response initiates secondary changes in the injured neighbouring tissues and this whole physiological response in the tissues is called as the process of inflammation (Guyton & Hall, 2006). Inflammation is the protective response of the body which is participated by blood vessels, host cells, proteins and different type of mediators. This protective effect is carried out by initially diluting, destroying or neutralizing the injurious agents (Kumar et al., 2012). The function of inflammation is to combat the initial source of the injury done to the cells or tissues to provide defense against necrosis and to eradicate various cells or tissues damaged during this process. Inflammation also induces repairing of the damaged tissues after the initial insult. The area where the inflammation is going on is marked with swelling, redness, pain and heat sensation. Inflammation is the natural response of the body and referred to as the mechanism of innate immunity (Abbas et al., 2012). The ability of the body to induce inflammation is necessary for its survival against

various foreign pathogens and different kind of injuries evoked by various stimuli like physical injuries, antibodies, various pathogens and different kind of infections. The inflammatory process during normal conditions and in various diseased states may be induced and maintained without any beneficial effects or may cause some noxious consequences (Laurence et al., 2008). The pathophysiology of inflammation involves the excessive blood flow to the damaged tissues due to increased vasodilation of blood vessels. Inflammation is also characterized by the movement of a large number of leukocytes in to the effected tissues. Due to increased permeability of the capillaries there is excessive leakage of fibrinogen which results in the formation of fluid clotting in interstitial spaces. Various inflammatory mediators stimulate the immune system. Thus, with in a period of only a few hours the macrophages start engulfing the damaged tissues. But during this defensive process they further damage the neighbouring normal tissues (Guyton & Hall, 2006). During the process of inflammation there is an initial inflammatory response to different inflammatory mediators and the second one is the healing process of the injured tissues. So, these steps cause considerable damage to the tissues. Immune system and inflammation are the defensive mechanisms of the body and both are interrelated to each other. Inflammation results from any immune reaction while activation of immune system is a necessary factor before the process of inflammatory response (Mohan, 2010). One of the most popular phenomenon of inflammation is the "walling off" the surrounding undamaged tissues from the injured tissues. Fibrinogen clots block the lymphatic and the interstitial spaces of the damaged area so after a shorter period of time there is an increased flow of fluids in these spaces. The advantage of walling-off mechanism is that it delays the spreading of various pathogens and different injurious products (Guyton & Hall, 2006). Inflammation is

characterized by three specific basic phases with each phase involving different mechanisms. The initial one is the acute phase of inflammation where an increased vasodilation and enhanced permeability of the capillaries occurs. The second one is the subacute phase with a delayed inflammatory response that involves migration of different phagocytic cells and leukocytes to the inflamed area. Chronic proliferative phase is the third and last phase which is characterized by degeneration of the tissues. Fibrosis also takes place in this stage (Laurence et al., 2008). Without the process of inflammation there will be no monitoring of the infections and will worsen with the passage of time. Secondly, without inflammation there will be no healing process (Kumar et al., 2012). There are two types of inflammation namely acute and chronic inflammation as discussed below.

### 1.5.1 Acute inflammation

Acute inflammation is characterized by the rapid transport of leukocytes and plasma proteins to the injurious area. Once the leukocytes are migrated at the site of injury they start clearing the area from the invaders and start engulfing and removing the necrotic tissues (Kumar et al., 2012). The duration of acute inflammation is very short which may be less than a period of two weeks. Acute inflammation is typified by plasma and fluid accumulation at the area of injury, platelet activation and neutrophil infiltration. Sometime the response of acute inflammation is more serious and that is why it is called fulminant acute inflammation (Mohan, 2010). Acute inflammation is divided in to two events, vascular and cellular changes.

### 1.5.1 (a) Vascular changes

It involves the changes in the vascular capabilities which cause increased vasodilation. Vascular changes are also characterized by the enhanced permeability of the capillaries which results in the leakage of plasma proteins from the blood vessels. Furthermore, vascular changes are accompanied by stimulating endothelial cells as a result of this activation leukocytes start adhering to these endothelial cells and starts rolling and migration along the blood vessels wall (Kumar et al., 2012).

### 1.5.1 (b) Cellular events

Vascular changes are followed by cellular events which are characterized by the infiltration of leukocytes from the wall of blood vessels. After this they enter into the injury zone and start gathering in large numbers over there. The gathered leukocytes are activated and they start the removal of invaded harmful agents. The major leukocytes that participate in the acute inflammation are polymorphonuclear leukocytes (neutrophils) (Kumar et al., 2012).

Various mediators can induce the response of acute inflammation. Amongst them viral, bacterial, parasitic and fungal infections are more common triggers. Moreover, different kind of foreign bodies like dirt, crystal deposits and splinters etc can also induce acute inflammation. Different chemical substances, physical trauma or certain environmental factors may also induce acute inflammation. Numerous conditions of ischemia are also responsible for the induction of acute inflammation (Kumar et al., 2012).