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Girls College for Arts, Science & Education
Zoology Department

*Asymmetrical Dimethylarginine (ADMA) as a Risk
Factor in Hypertension
Associated with Adriamycin Nephrosis in the Rats:
the Prophylactic Role of Arginine Treatment*

*For the degree of
Ph. D. of Science in Zoology*

By

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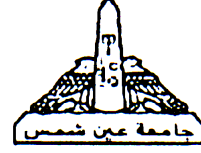
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جامعة عين شمس
كلية النبات للآداب و العلوم و التربية
قسم علم الحيوان

**أرجنين ثنائي المثيل غير المتماثل كعامل خطر في ارتفاع ضغط الدم
المصاحب لالتهاب الكلى في الجرذان بواسطة الأدراميسين: الدور
الوقائي للعلاج بالأرجنين**

رسالة مقدمة من

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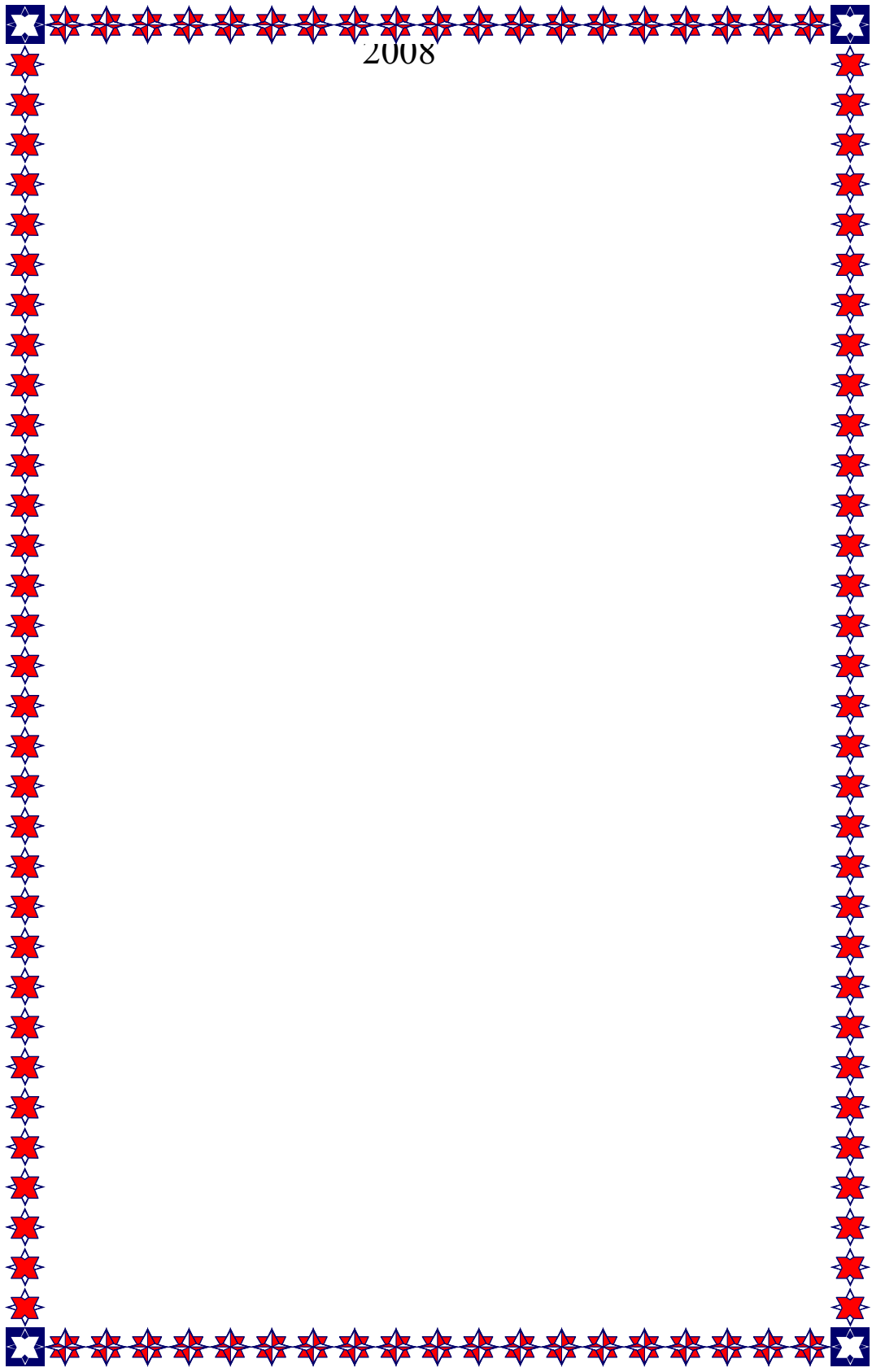
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Abstract

This study is an attempt to investigate the physiological and biochemical role of plasma ADMA as a result of induction hypertension or/and renal failure in male adult albino rats.

Furthermore, the current investigation focused on the therapeutic role of the administration of L-arginine on hypertensive rats to reduce or cure the hazard effects of hypertension problems.

Results were obtained from the treated experimental animal groups and compared with the corresponding normal control rat group.

The present findings were further discussed in view of relevant literature available in similar fields of studies.

The following parameters were estimated:

Cardiac profile, lipid profile, kidney function profile, lipid peroxidation and asymmetric dimethylarginine (ADMA).

It is concluded from the results of the present investigation that exposure to adriamycin increasing in ADMA level which caused a dysfunction of the endothelial L-arginine/ nitric oxide pathway which is conceder a common mechanism by which several cardiovascular risk factors mediate certain deleterious effects on the vascular wall such as hypertension in male albino rats. On the other hand, L-arginine administration lessened or cured these damages and repaired the destruction which occurred as a result of adriamycin supplementation. In addition suggestions for further studies were recommended.

Key words:

Hypertension – renal failure – ADMA – adriamycin – arginine.

Introduction

In many economically developing countries, such as Egypt, patterns of illness are changing dramatically. Specifically, communicable diseases are becoming less common, and the incidence and prevalence of non communicable chronic diseases, such as hypertension, are rising (*Nissenen et al., 1988; Kaplan, 2002 and Oparil & Weber, 2006*). Indeed, several lines of evidence suggest that hypertension and its complications are a major health problem in Egypt. First, the incidence of blood pressure (BP) related clinical events such as myocardial infarction, stroke, and end-stage renal disease appears to be increasing dramatically. Vital statistics (such as cause-specific mortality and hospitalization discharge diagnoses) along with clinical observations support this notion (*Dayem et al., 1978*). Second, preliminary surveys suggest that the prevalence of hypertension may be as high as 26.3% of Egyptian adults (≥ 25 years) (*Ibrahim et al., 1995*).

Although the first measurement of (BP) was performed in 1711 when Reverend Stephen Hales cannulated the carotid artery of a horse, extensive animal and clinical experimentation to the mechanisms responsible for the normal regulation of arterial pressure and development of essential or primary hypertension remain unclear. Riva-Rocci (1896) and Korotkov (1904) allowed the measurement of branchial systolic and diastolic blood pressure in humans. In 1911, Frank introduced the term “essential hypertonie”, which became essential hypertension and this term is now used to indicate hypertension of unknown etiology. For over 100 years physicians have studied the relationship between elevated blood pressure and vascular disease. Nowadays, the study of structural and mechanical alterations of small arteries as well as their possible role in the pathogenesis of essential hypertension still remains a matter of clinical and scientific interest (*Guyton, 1991*;

Cowley, 1992; Navar, 1997; Oparil & Weber 2000; Topol, 2002; Zervoudaki & Toutouzas 2003 and Goldman & Ausiello, 2004).

The improved control of infectious and parasitic diseases and the sharp decrease in infant mortality during recent decades has dramatically changed the health profile of many economically developing countries (*Ganong, 1991 and Jamison et al., 1993*). This is reflected in a prolongation of life expectancy and the emergence of cardiovascular disease as a leading cause of morbidity and mortality in such countries (*Whelton et al., 1995*). Statistics from the Egyptian Ministry of Health and the Egyptian *Central Agency for Public Mobilization and Statistics (CAPMAS, 1990)* indicate that between 1960 and 1990 the average life expectancy of Egyptians increased from 51.6 to 62.8 years for men and from 53.8 to 66.4 years for women. In 1970, cardiovascular disease accounted for 12.4% of all deaths in Egyptians, whereas two decades later it was responsible for 42.5% of the nation's mortality.

The steady growth in the number of elderly patients with hypertension in Egypt means that the country will face a serious national problem by the year 2020, when it can expect to deal with 18 million elderly hypertensives (*Hassan, 1996*).

Hypertension is a cause and consequence of chronic renal disease. It's considers as the second most common cause of end-stage renal disease, with diabetes mellitus being the first. Renal failure in patients with hypertension has many causes, including functional impairment secondary to vascular disease and hypertensive nephrosclerosis. Even in those in whom hypertension is not the primary process damaging the kidney, elevations in systemic blood pressure may accelerate the rate at which kidney function is lost (*Kakinuma et al., 1996; Wolf et al., 1997; Weir & Dworkin, 1998; Kaplan, 2002 and Goldman & Ausiello, 2004*).

Asymmetric dimethylarginine (ADMA) is a naturally occurring component of human blood plasma. It is formed as a

metabolic byproduct of continuous protein turnover in all cells of the body. More than one decade ago, ADMA was first reported to exert biological effects by inhibiting NO synthesis. Starting with this initial finding, the patho-physiological role of ADMA has subsequently been elucidated (*Vallance, et al., 1992; Rawal, et al., 1995; Boger et al., 2000; Dayal & Lentz, 2005 and Eid et al., 2006*).

ADMA may play a prominent role in the pathogenesis and in the progression of cardiovascular diseases – specifically atherosclerosis. The clinical role of ADMA as a marker of cardiovascular risk can be deduced from an increasing number of clinical studies that have demonstrated the presence of a statistically significant and independent relationship between ADMA and the incidence of major adverse cardiovascular events or death (*Vallance, et al., 1992; Rawal, et al., 1995; Achan, et al., 2003 and Zoungas et al., 2006*).

The kidneys are a likely focal point for exploring the physiological control of plasma ADMA because elevated ADMA has been often reported in association with renal failure (*Vallance, et al., 1992; Kielstein, et al., 2001; Wahbi, et al., 2001; Zoccali, et al., 2001 and Baylis, 2008*).

Adriamycin is one of the drugs that are used to induce hypertension in rat model it is also one of the chemotherapeutic drugs that are given as treatment for many forms of cancer but unfortunately, has many side effects such as chest pain, breathing difficulty, swelling of ankles or feet, irregular heart beat and low blood counts. Special attention must be given to the cardiotoxicity and nephrotoxicity persuaded by adriamycin that results in hypertension (*Gewirtz, 1999; Wendel et al., 2004; Johansson et al., 2006 and Richard & Annette, 2007*).

Antioxidants reduce the effect of dangerous oxidants by binding together with these harmful molecules, thereby decreasing their destructive power. Antioxidants can also help repair the

damage already sustained by cells (*Krinky, 1992; Rock et al., 1996 and Tandon et al., 2004*).

Arginine is a basic amino acid which is considered semi-essential. Although it can be synthesized by the body, it cannot be produced at rates sufficient to support growth and must therefore be ingested in the diet. L-arginine has many important biochemical functions, including immunoreactivity, enhancing release of growth hormones, and amino acid detoxification. L-arginine is also an important source for polyamine synthesis and is the only source of amino groups in the formation of creatine, which is involved in the regeneration of ATP (*Boger et al., 1997; Tenenbaum et al., 1998; Stanislavov & Nikolova, 2003 and Jan, 2006*).

Alterations in L-arginine bioavailability and subsequently NO synthesis have been linked to the pathogenesis of a wide range of cardiovascular and renal diseases (*Maxwell et al., 2000; Mendes et al., 2001 and Mendes Ribeiro & Brunini, 2004*).

Arterial hypertension and chronic renal and heart failure are pathological states with high morbidity and mortality, and the full understanding and adequate treatment of these diseases remain a challenge. Accumulating evidence indicates that a disturbance of the L-arginine-NO pathway is involved in the pathogenesis of these diseases (*Peters et al., 2001; Annuk et al., 2003; Anderson et al., 2004; Boger, 2007 and Baylis, 2008*).

Aim of the work

In the worldwide, hypertension remains a common and serious problem, contributing in a major way to the most common causes of morbidity and mortality in developed societies. Moreover, the main burdens associated with hypertension occur not in the relatively few with severe disease but in the masses of patients with BPs that are only minimally elevated (*Stamler et al., 1993 and Cruickshank et al., 2001*).

Therefore, this study was undertaken to investigate the physiological and biochemical role of plasma ADMA as a result of induction hypertension or/and renal failure in male adult albino rats. In addition, this work aimed to focus on the beneficial role of antioxidants (arginine) in retardation and regression of hypertension problems.

To evaluate these targets the following investigation had been undertaken:

- Detection of hazardous and perturbation of hypertension through detection of blood pressure measurement, biochemical parameters including cardiac profile, kidney function profile, lipid profile, lipid peroxidation and asymmetric dimethylarginine.
- Assessment of the therapeutic role of arginine supplementation in terms of lowering and correction the effects of hypertension using biochemical analysis.
- Discussion of the implied results in view of the relevant literature.

Review of the Literature

Hypertension

Hypertension, commonly referred to "high blood pressure", is a medical condition usually associated with narrowing of the arteries. This causes blood to be pumped with excessive force against the artery walls. It is called "Silent killer" because most people have no reason to think they might be hypertensive (*Guyton, 1991 and Navar, 1997*). While, it is formally called arterial hypertension, the word "hypertension" without a qualifier usually refers to arterial hypertension. Persistent hypertension is one of the risk factors for strokes, heart attacks, heart failure and arterial aneurysm, and is a leading cause of chronic renal failure (*Chobanian, 2003; Kim et al., 2005 and Sagnella & Swift, 2006*).

The role of hypertension probably is underestimated from morbidity and mortality statistics, which are largely based on death certificates. When a patient dies from a stroke, a heart attack, or renal failure all directly attributable to uncontrolled hypertension the stroke, the heart attack, or the renal failure, not the hypertension, usually is listed as the cause of death (*Kaplan, 2002 and Oparil & Weber, 2006*).

Probably no other field of clinical medicine enjoys a greater scientific base than hypertension. Increasingly detailed understanding of the basic mechanisms involved in blood pressure regulation and related metabolic disorders has led to the identification of a large list of effective medications as well as lifestyle modifications for hypertension. Randomized controlled trials have provided unequivocal proof that lowering blood pressure with medications dramatically reduces the risk of disability and death from cardiovascular and renal disease. However, high blood pressure remains untreated or inadequately treated in the majority

of affected individuals. As a consequence, high blood pressure still is the leading cause of death worldwide figure (1).

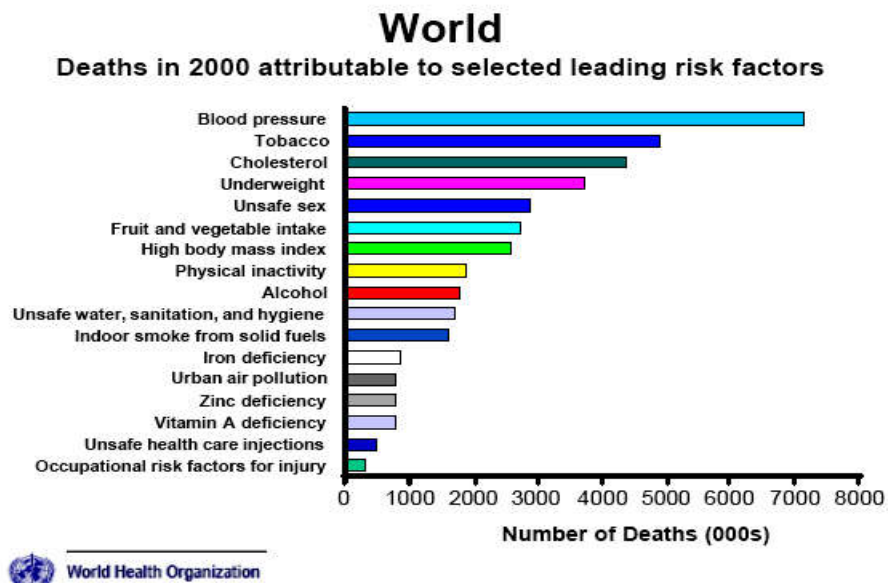


Figure (1): Blood pressure is the first cause of death world wide
(Quoted from *American Society of Hypertension, 2005*).

Hypertension Prevalence in Egypt

- Prevalence of hypertension is 26.3% of Egyptian adults (≥ 25 years).
- Age related prevalence:
 - Prevalence in the category (25 to 34-years old) is 7.8%,
 - Prevalence in the category (75 years or older) is 56.6%,
 - Peak of prevalence is in the category (55- to 64-years-old), which is 59.4%.
- Gender related prevalence: Prevalence in women is 26.9% and in men is 25.7%.
- Geographical related prevalence:
 - Cairo prevalence rate is (31%),
 - In rural areas the prevalence is (25%),
 - In frontiers (Oases) the prevalence is (19.9%) (*Ibrahim et al., 1995*).

Generally, the higher the blood pressure and the longer it remains elevated, the greater the morbidity and mortality. Untreated hypertension affects all organ systems and can shorten one's life expectancy by 10 to 20 years. The British Hypertension Society for hypertension management classify blood pressure as normal, pre-hypertension, hypertension stage 1, and hypertension stage 2 table (1).

CLASSIFICATION OF BLOOD PRESSURE			
CATEGORY	SBP MMHG		DBP MMHG
Normal	<120	and	<80
Prehypertension	120-139	or	80-89
Hypertension Stage 1	140-159	or	90-99
Hypertension Stage 2	≥160	and	≥100

Table (1): Classification of blood pressure (*Quoted from British Hypertension Society for hypertension management 2004*).

Hypertension can be classified as either essential or secondary. Essential hypertension is the term used when no specific medical cause can be found to explain a patient's condition. Secondary hypertension means that the high blood pressure is a result of (i.e. secondary to) another condition, such as kidney disease (*Wing et al., 2003 and Johnson & Turner, 2005*).

Essential hypertension: is a far more common condition and accounts for 95% of the population with hypertension. No specific cause for the elevated blood pressure can be identified. Nevertheless, certain associations have been recognized in people with essential hypertension. Salt intake may be a particularly important factor in relation to essential hypertension in a number of varied situations. This is associated with advancing age, black racial background, obesity and hereditary (genetic) susceptibility (*Goodman, 1992; Luke, 1993; Rettig et al., 1993; Hen et al., 1998; Kaplan, 2002 and Williams et al., 2004*).

Secondary hypertension: however account for 5% of all cases where the high blood pressure is secondary to or caused by a specific abnormality in one of the organs or systems of the body such as the kidney, adrenal gland, or aortic artery (*Ganong, 1991; Ploth & Navarr, 1993; Kaplan, 2002 and Mansoor, 2004*).

Okuda et al. (1986) reported that the hypertensive rats where hypertension was induced with adriamycin, led to a marked elevation in the body weight and a significant depletion in the kidney weight. The authors attributed these results to the abnormal retention of sodium and pharmacological nephropathy of adriamycin.

At the beginning of blood pressure elevation, oedema is developed in men and women especially in pregnant women and lead to toxemia (pre-eclampsia and eclampsial). Toxaemic pregnant women are accompanied with increase in their body weight due to water retention (*Scott, 1990*).

Chronic high blood pressure can also lead to an enlarged heart, kidney failure and an increased resistance in the peripheral arteries throughout the tissues of the body causing a work load on the heart muscle to pump the blood through these blood vessels. Accordingly, a strain is put on the heart, which can lead to heart abnormalities that are usually first seen as enlarged heart muscle (*Goodman, 1992*).

Khalil (1996) pointed to that the steady growth in the number of patients with hypertension in Egypt means that the country will face a serious national problem by the year 2010, when it can expect to deal with 10 million hypertensives patients, and an even more serious one by the year 2020 when this number is projected to reach about 18 million.

Cai and Harrison (2000) supposed that most if not all risk factors that are related to atherosclerosis and cardiovascular morbidity and mortality, including traditional and nontraditional

risk factors, were also found to be associated with endothelial dysfunction. Many of these risk factors, including hyperlipidemia, hypertension, diabetes, and smoking are associated with overproduction of reactive oxygen species or increased oxidative stress.

Sharoud (2002) reported that the pre-eclampsia and eclampsia women suffered from oedema, proteinuria, platelet aggregation and elevation in the osmolarity of blood depending on their cases.

Vasan et al. (2002) demonstrated that hypertension affects approximately 50 million individuals in the United States and approximately 1 billion worldwide. As the population ages, the prevalence of hypertension will increase even further unless broad and effective preventive measures are implemented.

Rhadriguez-Gomez (2003) noticed that the administration of N-nitro-L-arginine methyl ester (L-NAME) led to elevation of blood pressure accompanied with increase in body weight due to oedema and a significant decrease in the kidney weight due to atrophy and injuries in the glomerular tubules.

Heibashy and Abdel Moneim (2005) studied that the increments in the levels of total nitric oxide and angiotensin II are responsible for an increase in the blood pressure and maintenance of hypertension through the stimulation of oxidative stress due to the injection with L-NAME. The authors recorded a significant decrease in the kidney weight of hypertensive rats due to the nephrotic syndrome accompanied with hypercholesterolemia and expansion of extravascular fluid volume.

Yang and Ming (2006) carried out that decreased bioavailability of endothelial nitric oxide produced from endothelial NO synthase, referred to as endothelial dysfunction, plays a crucial role in the development and progression of hypertension.