Original

UNIVERSITI SAINS MALAYSIA

RESEARCH UNIVERSITY (RU) GRANT TECHNICAL REPORT

Project Title	:	**The Effects Cross-Fostering And Melatonin Supplementation On The Oxidant/Antioxidant Status And Development Of Hypertension In Spontaneously Hypertensive Rats**
Investigator	:	**Assoc. Prof. Dr. K.N.S.SIRAJUDEEN**
Co-Researcher	:	**Professor Dr. Harbindar Jeet Singh**
Grant A/c No.	:	

JUNE 2011

RESEARCH UNIVERSITY GRANT FINAL REPORT

UNIVERSITI SAINS MALAYSIA

UNIVERSITY RESEARCH GRANT FINAL REPORT
Geran Penyelidikan Universiti Laporan Akhir

A.	**TITLE OF RESEARCH:** *Tajuk penyelidikan:* **The Effects Cross-Fostering And Melatonin Supplementation On The Oxidant/Antioxidant Status And Development Of Hypertension In spontaneously Hypertensive Rats**
B.	**PERSONAL PARTICULARS OF RESEARCHER** / *MAKLUMAT PENYELIDIK:*
(i)	**Name of Research Leader:** *Nama Ketua Penyelidik:* **Assoc.Prof. Dr. K.N.S.Sirajudeen**
	Name of Co-Researcher *Nama Penyelidik Bersama:* **Professor Dr. Harbindar Jeet Singh**
(ii)	**School/Institute/Centre/Unit :** *Pusat Pengajian /Institut/Pusat/Unit :* **School of Medical Sciences**

C. **Research Platform (Please tick (/) the appropriate box):**
Pelantar Penyelidikan (Sila tanda (/) kotak berkenaan):

- [ ] **A. Life Sciences** *Sains Hayat*
- [√] **B. Fundamental** *Fundamental*
- [ ] **C. Engineering & Technology** *Kejuruteraan & Teknologi*
- [ ] **D. Social Transformation** *Transformasi Sosial*
- [ ] **E. Information & Communications Technology (ICT)** *Teknologi Maklumat & Komunikasi*
- [ ] **F. Clinical Sciences** *Sains Klinikal*
- [ ] **G. Biomedical & Health Sciences** *Bioperubatan Sains Kesihatan*

D.	**Duration of this research :** *Tempoh masa penyelidikan ini :* ***Duration :** 3 years & 6 months *Tempoh :* **From :** 22/10/2007 **To** 21/03/2011 *Dari:* *Ke :*

Pejabat Pelantar Penyelidikan 2009

E. **ABSTRACT OF RESEARCH**

(An abstract of between 100 and 200 words must be prepared in **Bahasa Malaysia and in English**. This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

This study examined the effects of either cross-fostering or melatonin supplementation alone or in combination on the development of hypertension and renal antioxidant/oxidant system in SHR and WKY rats. One-day-old offspring of SHR and WKY dams received melatonin (10mg/kg body weight) from gestation day-1 cross-fostered to opposite strain of dam and these male offspring continued to receive melatonin (10mg/kg body weight) up to 16 weeks of age. Systolic blood pressure (SBP) of male offspring was recorded at the age of 4, 6, 8, 12 and 16 weeks, and the kidneys were collected for the estimation of antioxidant/oxidant status at the end of each age category.

In cross-fostered SHR, SBP was significantly lower than that in SHR controls till the age of 12 weeks and TBARS was lower at 4 weeks. SBP in melatonin treated SHR offspring remained significantly lower till the age of 16 weeks when compared to untreated SHR. GPx and GST activities were significantly higher at the age of 16 weeks, and total glutathione level was significantly higher at 4, 12 and 16 weeks in melatonin treated SHR when compared to untreated SHR. In melatonin supplemented cross-fostered SHR, SBP was significantly lower at the age of 12 and 16 weeks when compared to untreated cross-fostered SHR, but was significantly lower throughout the experimental period when compared to untreated in-fostered SHR. Activities of GPx and GST were higher in melatonin-treated cross-fostered SHR aged 16 weeks, when compared to age-matched SHR and non-melatonin supplemented cross-fostered SHR.

This study suggests that both melatonin supplementation and cross-fostering reduce the rate of rise in SBP. A combination of melatonin supplementation and cross-fostering however did not confer any additional impact on the blood pressure lowering effect of either melatonin or cross-fostering alone.

Abstrak Penyelidikan

(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Ingge** Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu ca untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

Kajian ini memeriksa kesan peliharaan silang, pemberian melatonin semasa antenatal dan postnatal atau kombinasi kedua-duanya ke atas perkembangan hipertensi dan sistem antioksidan/oksidan ginjal dalam tikus SHR dan WKY. Anak daripada ibu WKY dan SHR yang diberi melatonin (10mg/kg berat badan) dipelihara silang dari usia satu hari sehingga bercerai susu dan pemberian melatonin (10mg/kg berat badan) diteruskan kepada anak tikus jantan yang telah bercerai susu sehingga berusia 16 minggu. Tekanan darah sistolik (SBP) anak tikus jantan dicatat pada usia 4, 6, 8, 12 dan 16 minggu serta tisu ginjalnya dipungut untuk menganggar status antioksidan/oksidan pada setiap penghujung kajian menurut kategori usia.

SBP mencatatkan penurunan secara signifikan dalam SHR yang dipelihara oleh ibu WKY berbanding kawalan sehingga usia 12 minggu. TBARS didapati lebih rendah secara signifikan dalam SHR yang dipelihara oleh ibu WKY pada usia 4 minggu berbanding dengan kawalan berpadankan usia masing-masing. SBP dalam anak SHR dirawat melatonin menunjukkan penurunan signifikan sehingga usia 16 minggu. Aktiviti GPx dan glutation s-transferase (GST) dalam SHR yang menerima melatonin sehingga usia 16 minggu adalah lebih tinggi secara signifikan pada usia 16 minggu, dan tahap glutation keseluruhan adalah lebih tinggi secara signifikan pada usia 4, 12 dan 16 minggu berbanding dengan SHR yang tidak dirawat.

SBP adalah lebih rendah secara signifikan hanya pada usia 12 dan 16 minggu dalam SHR yang diperlihara oleh ibu WKY dan diberi melatonin sepanjang tempoh eksperimen berbanding dengan SHR yang dipelihara oleh ibu WKY dan tidak dirawat, tetapi SBP adalah lebih rendah secara signifikan sepanjang tempoh eksperimen dalam SHR yang tidak dirawat sama ada dipelihara oleh ibu SHR atau WKY. Aktiviti GPx dan GST mencatatkan peningkatan signifikan dalam SHR yang dipelihara oleh WKY dan diberi melatonin pada usia 16 minggu jika dibandingkan dengan SHR yang tidak dirawat sama ada dipelihara oleh ibu SHR atau WKY.

Sebagai kesimpulan, kajian ini mencadangkan pemberian melatonin dan peliharaan silang dapat menurunkan kadar kenaikan SBP. Walau bagaimanapun, kombinasi pemberian melatonin dan peliharaan silang tidak menunjukkan sebarang kesan tambahan terhadap penurunan tekanan darah jika dibandingkan dengan pemberian melatonin atau peliharaan silang secara berasingan.

F. **SUMMARY OF RESEARCH FINDINGS**

Ringkasan dapatan Projek Penyelidikan

- This study suggests that the major abnormality in SHR lies in the CAT and GPx, both involving the H_2O_2 detoxification system.
- Cross-fostering delays the development of hypertension in SHR offspring, but its hypotensive effect is not via the restoration in renal antioxidant/oxidant parameters measured in this study.
- Antenatal and postnatal melatonin supplementation suppresses the rise in blood pressure in SHR offspring, but not to the normotensive level as found in WKY rats.
- A combination of antenatal and postnatal melatonin supplementation and cross-fostering does not have any additional hypotensive effect and does not restore the raised blood pressure in SHR offspring to the normotensive levels.

G. **COMPREHENSIVE TECHNICAL REPORT**

Laporan Teknikal Lengkap

Applicants are required to prepare a comprehensive technical report explaining the project.
(This report must be attached separately)
Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.
[Laporan ini mesti dikepilkan]

List the key words that reflectour research:

Senaraikan kata kunci yang mencerminkan penyelidikan anda:

English	Bahasa Malaysia
Spontaneously hypertensive rats	tikus hipertensi spontan
Melatonin and Cross- fostering	melatonin dan peliharaan silang
Systolic blood pressure and Renal oxidant-antioxidant status	tekanan darah sistolik dan oxidan-antioxidan ginjal

H.

a) Results/Benefits of this research
Hasil Penyelidikan

No. *Bil:*	Category/Number: *Kategori/ Bilangan:*	Promised	Achieved
1.	**Research Publications (Specify target journals)** *Penerbitan Penyelidikan (Nyatakan sasaran jurnal)*	2	3 (full paper) + 2 (Journal abstracts) + 8 Conference presentations
2.	**Human Capital Development**		
	a. Ph. D Students	**1**	**1**
	b. Masters Students		
	c. Undergraduates (Final Year Project)		
	d. Research Officers		
	e. Research Assisstants		
	f. Other: Please specify		
3.	**Patents** *Paten*	-	-
4.	**Specific / Potential Applications** *Spesifik/Potensi aplikasin*	-	-
5.	**Networking & Linkages** *Jaringan & Jalinan*	-	Co-researcher at UiTM
6.	**Possible External Research Grants to be Acquired** *Jangkaan Geran Penyelidikan Luar Diperoleh*	-	-

- Kindly provide copies/evidence for Category 1 to 6. **(Attached in the final report)**

b) Equipment used for this research.
Peralatan yang telah digunakan dalam penyelidikan ini.

Items *Perkara*	Approved Equipment	Approved Requested Equipment	Location
Specialized Equipment Peralatan khusus	Spectrophotometer Real-time Thermocycler ELISA reader	Master Cycler Pro S, Eppendorff,Germany --	Department of Chemical pathology, PPSP Central Research Lab (CRL), PPSP
Facility Kemudahan	Rat BP instrument & Animal hourse facilities	-	LARU, Health Campus,USM
Infrastructure Infrastruktur	General Lab equipment	-	CRL, Dept. Of Physiology & Dept. Of Chemical Pathology, PPSP

- Please attach appendix if necessary.

I. **BUDGET** / *BAJET*

Perbelanjaan :Expenditure

Project Account No.	: 1001/PPSP/811018
Total Approved Budget	: RM 159,000
Total Additional Budget	: RM 39,000
Grand Total of Approved Budget	: RM 198,000.00

Yearly Budget Distributed

Year 1	: RM 69,000
Year 2	: RM 43,900
Year 3	: RM 46,100

Additional Budget Approved

Year 1	: RM ----
Year 2	: RM 39,000
Year 3	: RM ----

Total Expenditure	: RM 197,938.05
Balance	: RM 61.95

- **Please attach final account statement from Treasury**

30/6/2011

Signature of Researcher
Tandatangan Penyelidik

Date
Tarikh

H. **COMMENTS OF PTJ'S RESEARCH COMMITTEE**
KOMEN JAWATANKUASA PENYELIDIKAN PERINGKAT PTJ

General Comments:
Ulasan Umum:

Project completed with objectives achieved. The output include ISI publication (publication).

Good output for research

Signature and Stamp of Dean/ Director of PTJ
Tandatangan dan Cop Dekan/ Pengarah PTJ

Dekan
Pusat Pengajian Sains Perubatan
Kampus Kesihatan
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan.

Date : 19/7/14.
Tarikh :

UNIVERSITI SAINS MALAYSIA

JABATAN BENDAHARI

KUMPULAN WANG UNIVERSITI PENYELIDIKAN (RU)

PENYATA PERBELANJAAN SEHINGGA 30 JUN 2011

	RM		
Jumlah Geran :	198,000.00	Ketua Projek :	PM DR K.N.S Sirajudeen
Peruntukan OKT 2007 (Tahun 1)	69,000.00	Tajuk Projek:	The Effects Cross-Fostering And Melatonin Supplementation On The Oxidant/Antioxidant Status And Development Of Hypertension In spontaneously Hypertensive Rats
Peruntukan OKT 2008 : (Tahun 2)	43,900.00	Tempoh :	3 Tahun (22/10/2007-21/09/2010)
TAMBAHAN 1	39,000.00	T. Lanjut :	22/09/2010-21/03/2011
Peruntukan OKT 2009 : (Tahun 3)	46,100.00	No. Akaun :	1001/PPSP/811018

Kwgan	Akaun	PTJ	Projek	Peruntukan Projek	Perbelanjaan Terkumpul sehingga Tahun lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
1001	11000	PPSP	811018	30,000.00		30,000.00			-	30,000.00
1001	14000	PPSP	811018			-			-	-
1001	15000	PPSP	811018			-			-	-
1001	21000	PPSP	811018		19,631.77	(19,631.77)			-	(19,631.77)
1001	22000	PPSP	811018			-			-	-
1001	23000	PPSP	811018			-			-	-
1001	25000	PPSP	811018			-			-	-
1001	26000	PPSP	811018			-		140.00	140.00	(140.00)
1001	27000	PPSP	811018	97,600.00	96,057.18	1,542.82		30,247.80	30,247.80	(28,704.98)
1001	28000	PPSP	811018			-			-	-
1001	29000	PPSP	811018	6,400.00	2,367.20	4,032.80	450.00	44.10	494.10	3,538.70
1001	32000	PPSP	811018			-			-	-
1001	35000	PPSP	811018	64,000.00	49,000.00	15,000.00			-	15,000.00
				198,000.00	167,056.15	30,943.85	450.00	30,431.90	30,881.90	61.95

TABLE OF CONTENTS

ACKNOWLEDGEMENT

This project was carried out under the financial support of USM Research University Grant (1001 / PPSP / 811018). We would like to thank the Central Research Laboratory (CRL), Department of Physiology & Department of Chemical Pathology, School of Medical Sciences and Laboratory Animal Research Unit (LARU), Health campus, Universiti Sains Malaysia for providing the facilities to carry out the study. The contribution of Ms. Lee siew keah (Ph.D student) in carrying out the study is very much appreciated.

ABSTRACT

This study examined the effects of either cross-fostering or melatonin supplementation alone or in combination on the development of hypertension and renal antioxidant/oxidant system in SHR and WKY rats. One-day-old offspring of SHR and WKY dams received melatonin (10mg/kg body weight) from gestation day-1 cross-fostered to opposite strain of dam and these male offspring continued to receive melatonin (10mg/kg body weight) up to 16 weeks of age. Systolic blood pressure (SBP) of male offspring was recorded at the age of 4, 6, 8, 12 and 16 weeks, and the kidneys were collected for the estimation of antioxidant/oxidant status at the end of each age category.

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ABSTRAK

Kajian ini memeriksa kesan peliharaan silang, pemberian melatonin semasa antenatal dan postnatal atau kombinasi kedua-duanya ke atas perkembangan hipertensi dan sistem antioksidan/oksidan ginjal dalam tikus SHR dan WKY. Anak daripada ibu WKY dan SHR yang diberi melatonin (10mg/kg berat badan) dipelihara silang dari usia satu hari sehingga bercerai susu dan pemberian melatonin (10mg/kg berat badan) diteruskan kepada anak tikus jantan yang telah bercerai susu sehingga berusia 16 minggu. Tekanan darah sistolik (SBP) anak tikus jantan dicatat pada usia 4, 6, 8, 12 dan 16 minggu serta tisu ginjalnya dipungut untuk menganggar status antioksidan/oksidan pada setiap penghujung kajian menurut kategori usia.

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SBP adalah lebih rendah secara signifikan hanya pada usia 12 dan 16 minggu dalam SHR yang diperlihara oleh ibu WKY dan diberi melatonin sepanjang tempoh eksperimen berbanding dengan SHR yang dipelihara oleh ibu WKY dan tidak dirawat, tetapi SBP adalah lebih rendah secara signifikan sepanjang tempoh eksperimen dalam

SHR yang tidak dirawat sama ada dipelihara oleh ibu SHR atau WKY. Aktiviti GPx dan GST mencatatkan peningkatan signifikan dalam SHR yang dipelihara oleh WKY dan diberi melatonin pada usia 16 minggu jika dibandingkan dengan SHR yang tidak dirawat sama ada dipelihara oleh ibu SHR atau WKY.

Sebagai kesimpulan, kajian ini mencadangkan pemberian melatonin dan peliharaan silang dapat menurunkan kadar kenaikan SBP. Walau bagaimanapun, kombinasi pemberian melatonin dan peliharaan silang tidak menunjukkan sebarang kesan tambahan terhadap penurunan tekanan darah jika dibandingkan dengan pemberian melatonin atau peliharaan silang secara berasingan.

INTRODUCTION

Hypertension is a major risk factor contributing to cardiovascular, cerebrovascular and renal diseases, which together contribute to the high mortality rate worldwide. It is estimated that there are 4.8 million individuals with hypertension in Malaysia (Ministry of Health Malaysia., 2008). The third National Health and Morbidity Survey indicates a prevalence of hypertension among adults aged 30 years and above as 42%. From the survey it is alarming to note that close to two thirds of individuals with hypertension in Malaysia were unaware that they were hypertensive and therefore remained undiagnosed (Ministry of Health Malaysia., 2008). Hypertension is often referred to as a silent killer as it remains symptomless and is often only detected co-incidentally or during a routine medical examination. It is therefore important that hypertension is diagnosed early and well managed as its early and proper management has been shown to significantly reduce complications like strokes, myocardial infarction, congestive heart failure and end-stage renal disease (Collins and MacMahon, 1994; Arguedas *et al.*, 2009; de Lusignan *et al.*, 2009; Stenvinkel, 2010).

Hypertension is generally classified as either primary or secondary hypertension. The etiology and pathogenesis of primary or essential hypertension remain unidentified and under intense study. Essential, primary, or idiopathic hypertension is defined as high blood pressure in which secondary causes such as renovascular disease, renal failure, pheochromocytoma, aldosteronism, or other causes of secondary hypertension are not present (Carretero and Oparil, 2000). On this basis essential hypertension accounts for 95% of all cases of hypertension (Carretero and Oparil, 2000). It is considered a heterogeneous disorder, with different

patients having different causal factors that lead to high blood pressure. Although the causes of essential hypertension remain largely unknown, some information is available indicating the role of some genetic variations and intermediary phenotypes that might cause or be responsible for the high blood pressure. Existing evidence suggests that the genetic contribution to blood pressure variation is about 30% (Hong *et al.*, 1994; Marteau *et al.*, 2005), with the rest coming from a number of environmental factors that have also been linked to raised blood pressure, including obesity, insulin resistance, high salt intake, high alcohol intake, stress, aging, sedentary lifestyle, low potassium and low calcium intake (Hashimoto *et al.*, 1989; Sever and Poulter, 1989; Elliott *et al.*, 1990; Andrade *et al.*, 2010; Fujita and Takei, 2010).

An imbalance in the antioxidant/oxidant status especially in the kidney has been proposed as an important pathogenic mechanism in hypertension as well as the progression of kidney disease (Wilcox, 2005; Nistala *et al.*, 2008). In addition to this, maternal influence during early life has also been proposed and might be one of the risk factors contributing to the adult hypertension (Blizard and Adams, 1992; Ashton, 2000; Davidge *et al.*, 2008). In this regard, several experimental hypertension models e.g. spontaneously hypertensive rats, Dahl's, Milan, Lyon, deoxycorticosterone acetate-salt hypertensive, Sabra and New Zealand strains, differing in the contribution of genetic and environmental factors to the raised blood pressure, have been developed in attempts to understand the pathogenesis of hypertension (Okamoto and Aoki, 1963; Kihara *et al.*, 1993; Johns *et al.*, 1996). The spontaneously hypertensive rat (SHR), the closest animal model that represents human essential hypertension, and its Wistar-Kyoto (WKY) normotensive control were developed in 1963 by Okamoto and Aoki in Japan (Okamoto and Aoki, 1963). SHR exhibits spontaneous

hypertension with many features in common with human essential hypertension, which include elevated peripheral resistance, increased cardiac output, elevated sympathetic nervous activity and cardiovascular hypertrophy (Frohlich, 1986; Zicha and Kunes, 1999; Girouard *et al.*, 2004). Furthermore, as in human, its blood pressure is readily lowered with peripheral vasodilators, calcium channel antagonists and blockers of the renin-angiotensin system (Zicha and Kunes, 1999; Polizio and Pena, 2005; Liskova *et al.*, 2010). Numerous sophisticated attempts have been made to modify the natural course of hypertension in adult SHR with established hypertension via various pharmacological and nutritional interventions (Zicha and Kunes, 1999; Nava *et al.*, 2003; Rodriguez-Iturbe *et al.*, 2003; Khanna *et al.*, 2008; Nuyt and Alexander, 2009). In contrast, relatively little attention has been devoted to studies involving pre-weaning or young SHR in a bid to prevent the rise in blood pressure. Modification of pre-weaning maternal environment through cross-fostering of one-day-old SHR offspring to normotensive dams e.g have shown that cross-fostering significantly delayed the development of high blood pressure in these SHR offspring (Cierpial and McCarty, 1987; McCarty and Tong, 1995; Di Nicolantonio *et al.*, 2006). Although the precise reason for this delay in the rise in blood pressure was not evident, it was however attributed to differences in quality and quantity of milk delivered to the offspring and perhaps due to exposure of the SHR offspring to a different pattern of maternal behaviour (McCarty and Tong, 1995; Gouldsborough *et al.*, 1998). Whilst the impact of cross-fostering is only temporary, its influence nevertheless needs to be examined further to identify the factor responsible for the hypotensive effect and possibly also the mechanism by which the blood pressure is lowered. In this regard, the effect of cross-fostering on the renal antioxidant/oxidant status in SHR has not been investigated thoroughly and efforts could be made to examine the

mechanism and possibly also identify the particular factor in renal antioxidant/oxidant system.

A number reports over the years have documented a possible link between melatonin and the pathogenesis of hypertension. Decreased melatonin levels have been reported in hypertension (Jonas *et al.*, 2003; Leibowitz *et al.*, 2008), and melatonin supplementation has been shown to successfully ameliorate or reduce the high blood pressure in humans as well as in experimental animal models (Cagnacci *et al.*, 2005; Pechanova *et al.*, 2007). Clinical utility of melatonin in antenatal, parturition and postnatal life has been claimed to result in a wide range of health benefits, improved quality of life and reduction of complications during the neonatal period (Gitto *et al.*, 2009). Maternal melatonin treatment has been reported to reduce the raised blood pressure in offspring of genetically hypertensive animals (Kim *et al.*, 2002). Nevertheless, the association between its hypotensive and antioxidative effects and the regulation of renal antioxidant/oxidant system remains uncertain, particularly when administered during the antenatal, perinatal and postpartum periods. This study attempts to examine the impact of melatonin supplementation and cross-fostering either alone or in combination on the development of high blood pressure and renal antioxidant system in SHR.

Table 1 Principal experimental groups

Group	Description	Legend
A	WKY offspring reared by WKY dam	wky-WKY or WKY
B	WKY offspring reared by SHR dam	wky-SHR
C	WKY offspring reared by WKY dam, both melatonin supplemented	Mel-wky-WKY
D	WKY offspring reared by SHR dam, both melatonin supplemented	Mel-wky-SHR
E	SHR offspring reared by SHR dam	shr-SHR or SHR
F	SHR offspring reared by WKY dam	shr-WKY
G	SHR offspring reared by SHR dam, both melatonin supplemented	Mel-shr-SHR
H	SHR offspring reared by WKY dam, both melatonin supplemented	Mel-shr-WKY

Notes: Foetus received melatonin indirectly via placenta of the dam from gestation day-1 to the day of delivery. Neonates received melatonin indirectly from the milk of the foster dams starting on the day of birth until postnatal day-21. Weaned offspring received melatonin directly via drinking water from the day of weaning at postnatal day-21.

Determination of antioxidant activities/status and oxidative stress markers

Various antioxidants and oxidative stress markers were determined using the method/reagent kits as stated in Table 2.

Table 2: Determination of various antioxidant/oxidant levels

No.	Parameters	Methods/Reference
1.	SOD activity (U/mg protein)	Commercially available kit (Calbiochem, Germany)
2.	CAT activity (U/mg protein)	Goth et al (1991)
3.	GPx activity (U/mg protein)	Commercially available kit (Calbiochem, Germany)
4.	GR activity (U/mg protein)	Commercially available kit (Cayman, USA)
5.	GST activity (U/mg protein)	Commercially available kit (Cayman, USA)
6.	Total glutathione level (nmol/mg protein)	Commercially available kit (Sigma, USA)
7.	TAS (μmol/mg protein)	Koracevic et al (2001)
8.	TBARS level (nmol/mg protein)	Ohkawa et al (1979)
9.	PCO level (pmol/mg protein)	Commercially available kit (Cayman, USA)
10.	H_2O_2 level (μM)	Commercially available kit (Invitrogen, USA)

Determination of antioxidant protein level (GPx-1, GST-M1, CAT) by using Western blot analysis

10% kidney homogenate contained protease inhibitor cocktail was prepared. Samples were subjected into protein electrophoresis via SDS-PAGE. Separated proteins were transferred onto nitrocellulose membrane. Target protein was detected with appropriate primary and secondary antibody. The tagged protein was visualized using ECL system.

Determination of antioxidant mRNA level (GPx-1, GST-M1, CAT) by using Real-Time PCR methods

Total RNA was extracted using RNAeasy kit (Qiagen, Germany). After that, complementary DNA (cDNA) was synthesized using cDNA synthesis kit (Fermentas, USA). The cDNA was used as the DNA template for the real-time PCR reaction using SYBR green system (Stratagene, USA).

Statistical Analysis

Normal distribution and the homogeneity of variance of all the measured parameters were checked with Normality test and Levene's test. The data in this study met the assumptions for parametric statistical analysis i.e. data of each parameter was normally distributed with the equal variance. Therefore, data were analyzed using One-Way Analysis of Variance test (ANOVA) for multiple comparisons and followed by Post-Hoc Tukey Studies if significant differences were found among groups. Independent-samples t test was used to analyze between two different groups. Pearson's correlation test was applied to find a correlation between two variables. All the data was analyzed using statistical tests contained in the Statistical Package for the Social Science (SPSS) software version 15. Significance level was set at $p<0.05$. Data are expressed as mean and standard error of the mean (mean $\pm$ S.E.M.).

RESULTS & DISCUSSION

SBP, Body Weight and Renal Antioxidant/Oxidant status in SHR and WKY rats

Table 3 shows the changes in significance level of of SBP, Body Weight and Renal Antioxidant/Oxidant status of SHR vs WKY rats.

Table 3 SBP, body weight and renal antioxidant/oxidant status in SHR and age-matched WKY rats

Parameter/Age (weeks)	SHR vs WKY rats				
	4	6	8	12	16
SBP	NS	SHR↑	SHR↑	SHR↑	SHR↑
Body weight	SHR↓	SHR↓	SHR↓	SHR↓	SHR↓
SOD activity	NS	NS	NS	NS	NS
CAT activity	NS	SHR↑	SHR↑	SHR↑	SHR↑
CAT protein level	NS	SHR↑	SHR↑	SHR↑	SHR↑
CAT mRNA level	SHR↑	SHR↑	SHR↑	SHR↑	SHR↑
GPx activity	NS	NS	SHR↓	SHR↓	SHR↓
GPx protein level	NS	NS	NS	NS	NS
GPx mRNA level	NS	NS	NS	NS	NS
GR activity	NS	NS	NS	NS	NS
GST activity	NS	NS	NS	NS	NS
Total glutathione level	NS	NS	NS	NS	NS
TAS	NS	NS	NS	SHR↑	SHR↑
TBARS level	NS	NS	NS	NS	SHR↓
PCO level	NS	NS	NS	NS	NS
H_2O_2	NS	NS	SHR↓	SHR↓	SHR↓

NS: Non-significance
↑ Significantly higher, $p<0.05$
↓ Significantly lower, $p<0.05$

In general, the major findings of the present study are (i) CAT and TAS are elevated in SHR; (ii) GPx, TBARS, H_2O_2 are decreased in SHR; (iii) SOD, GR, GST, total glutathione and PCO levels in SHR are comparable to age-matched WKY rats. Overexpression of CAT mRNA is found from as early as 4 weeks of age, whereas

manifestation of its elevated protein level like increased enzymatic activity become evident at 6 weeks of age and onwards in SHR when compared to age-matched WKY rats. In contrast, lower GPx activity without significant changes in GPx-1 mRNA and protein level was found in SHR. Interestingly, H_2O_2 level was significantly lower in SHR. It appears that there is a disturbance in some of the AOE in SHR, which starts to show a significant difference from the normotensive WKY rats from the age of between 4 and 6 weeks, if not earlier, but before the rise in blood pressure. The discordance between GPx activity and its protein levels might either be due to reduced GPx substrate levels, or a selective inhibition in the activity of this enzyme, or an inherent abnormality in the GPx protein itself. Its decreased activity is less likely to be due to the availability of its co-factor GSH, since total glutathione, GR were comparable between SHR and WKY rats. The reason for the lower GPx activity therefore remains uncertain. GPx deficiency has been linked to endothelial dysfunction (Forgione *et al.*, 2002; Chrissobolis *et al.*, 2008), and atherosclerosis (Lewis *et al.*, 2007), which might in turn contribute to the pathogenesis of hypertension. Decreased GPx activity is expected to lead to H_2O_2 accumulation. However, interestingly, H_2O_2 in SHR was found to be significantly lower. It might therefore be speculated that (i) the abnormality lies with an increased local generation of H_2O_2 to begin with, which triggers the antioxidant defence system resulting in higher CAT activity but lower H_2O_2 levels, or (ii) a genetic abnormality in CAT overexpression which then results in excessive H_2O_2 decomposition or/and suppression of other AOE activities. With the former speculation, the increased H_2O_2 level might initially modify the active site on the GPx protein, which consequently leads to a loss of functional property of this antioxidant. As compensation, CAT

activity is up-regulated to minimize the increasing patho-physiological effects of reduced GPx activity.

It has been reported that normotensive volunteers with a family history of hypertension have been found to have higher plasma H_2O_2 levels than those normotensives without a family history of hypertension (Lacy *et al.*, 1998). This is unclear if younger SHR at the pre-hypertensive stage e.g. in embryo stage also have raised H_2O_2 as found in normotensive volunteer with genetically hypertensive background. Further investigations are required to examine the origin or the source of the elevated H_2O_2 to verify this hypothesis.

On the other hand, the abnormality might initially lie in the genetically abnormal overexpression of CAT, which may eventually suppresses the other AOE activities, especially those that share the same substrate (H_2O_2) with CAT i.e. GPx. Furthermore, overexpression of CAT may also lead to a negative feedback on its mRNA/protein expressions in later life. It has been demonstrated that 40-day-old SHR had raised myocardium CAT, however, its activity was found lower in SHR aged 19 months compared to age-matched WKY rats (Alvarez *et al.*, 2008). We suggest that the lower CAT in aged SHR might due to the negative feedback mechanism in its earlier up-regulated expressions. The beneficial effect of overexpression of CAT on prevention of the rise of blood pressure in SHR is not clear. Over activity of CAT in SHR has been reported previously, however, the data on its mRNA and protein expressions is very limited. This study proposed that its elevated activity may due to irregularities at the gene transcription level since the mRNA level was found significantly higher in younger SHR, and the raised mRNA subsequently contributes to the increased protein synthesis. We speculate that the raised CAT activity is the likely cause of the decreased H_2O_2 in SHR. Given the important