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EFFECTS OF ESSENCE OF CHICKEN ON IMMUNOLOGICAL RESPONSES AND BIOCHEMICAL CHANGES IN AN ANIMAL MODEL

By

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CERTIFICATE OF ORIGINALITY

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Yan Chun Man

Effects of Essence of Chicken on Immunological Responses and Biochemical Changes in an Animal Model

ABSTRACT

Aims

This study investigated the effects of essence of chicken (EOC) on immunological responses, free radicals and blood glucose concentrations in normal and burn wound rat models.

Methods

This thesis reports an experimental study on Sprague-Dawley rats with a 3-group time-series design: a sham operated group (UNBURN) (n=72), a burn inflicted group (BURN) (n=72) and a no treatment group (CONTROL) (n=24). Rats in the UNBURN and BURN groups were further subdivided by intake of normal saline (NS), gelatin (GEL) and essence of chicken (EOC) (n=24 each group). Blood samples were taken on days 1, 8, 15 and 22 postburn to measure concentrations of serum immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), plasma nitric oxide (NO), lipid peroxidation (LPO) and blood glucose. ANOVA was used for statistical analysis and the level of significance was set at $p<0.05$.

Results

Results demonstrate significant increases in serum IgG, IgA and IgM concentrations in both the GEL and EOC subgroups of the BURN and UNBURN groups in comparison to the placebo (NS) group on different postburn days. A significant

elevation of IgG was found in the GEL and EOC subgroups of the UNBURN group on days 1 ($p=0.026$) and 8 ($p=0.013$) respectively. Similar results were observed in the GEL ($p=0.001$) and EOC ($p=0.017$) subgroups of the BURN group on day 8. There was a significant increase in IgA in the GEL and EOC subgroups for both the BURN and UNBURN groups. In the EOC subgroups, IgA increased significantly on day 15 for the UNBURN group and day 22 for the BURN group ($p<0.050$ and $p=0.029$ respectively), whereas in the GEL subgroups, it was found to be higher on day 15 for the UNBURN group and day 22 for the BURN group ($p<0.050$ and $p=0.034$ respectively). Conversely, IgA dropped significantly in the GEL subgroup of the BURN group on days 1 and 15 ($p=0.038$ and $p=0.035$ respectively). A significant increase in IgM was observed in the EOC subgroups of both the BURN and UNBURN groups and that of the GEL subgroup in the UNBURN group. IgM increased significantly in the EOC subgroup of both the BURN and UNBURN groups, on day 15 ($p=0.040$) for the BURN group and days 1 and 22 ($p=0.010$ and $p<0.050$ respectively) for the UNBURN group, whereas in the GEL subgroup of the UNBURN group it was noted on day 22 only ($p=0.025$).

Blood glucose elevation was only observed in the EOC and GEL subgroups of the BURN group. Significant increases were noted in the EOC on days 1 and 22 ($p=0.045$ and $p=0.019$ respectively), whereas in the GEL subgroup, the increase was found on day 22 only ($p=0.019$). Similarly, significant changes in nitric oxide (NO) concentrations were also observed in the EOC and GEL subgroups of the BURN group only. However, there was a significant drop on day 15 for the EOC ($p=0.002$) and GEL subgroups ($p=0.024$). Concomitantly, a significant decrease in lipid peroxidation (LPO) was noted in the EOC subgroup of the BURN and UNBURN

groups (p=0.006 and p=0.018 respectively), whereas a similar result was only observed in the GEL subgroup of the BURN group (p=0.023) on day 8.

Conclusion

Results support the contention that EOC enhances serum immunoglobulin concentrations in both normal and burn-inflicted rats. The findings demonstrate that EOC suppressed NO and LPO concentrations in burned rats and that of LPO in normal rats. Therefore, EOC consumption may increase immunological response and prevent free radical-mediated diseases. Further, EOC has shown potential to regulate blood glucose after burn injury by maintaining a stable glucose supply for metabolic needs, which may facilitate wound healing.

SUMMARY OF CONFERENCE PRESENTATION AND PUBLICATIONS

Major parts of the materials presented in this thesis have formed the basis for papers that have been presented and submitted for publication as follows:

Conference Presentation

1. Yan, C. M., Chung W. Y., Wong, K. S., Tang, P. L., Wan, K. C. and Ko, K. K. (2004, August). *Effects of Essence of Chicken on Immunological Responses and Biochemical Changes in Animal Model*. Paper presented at the BRAND's 3rd Scientific Meeting, Bangkok: Thailand.

Publications

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LIST OF ABBREVIATIONS

%CV	=	Percent Coefficient of Variation
5-HIAA	=	5-hydroxyindolacetic acid
5-HT	=	5-hydroxytryptamine
ACTH	=	Adrenocorticotropic Hormone
ANOVA	=	Analysis of Variance
ATP	=	Adenosine Triphosphate
BEE	=	Basal Energy Expenditure
BHT	=	Butylated Hydroxytoluene
β-NADPH	=	β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
BSA	=	Bovine Serum Albumin
BUN	=	Blood Urea Nitrogen
BW	=	Body Weight
CD	=	Conjugated Dienes
CDS	=	Cluster Designation Systems
CH	=	Heavy Chain Constant Region
CIA	=	Collagen-induced Arthritis
CL	=	Light Chain Constant Region
CN^-	=	Cyanide
CNS	=	Central Nervous System
CO	=	Carbon Monoxide
CSF	=	Cerebrospinal Fluid
DA	=	Dark Agouti Rat

DOCA	=	Deoxycorticosterone Acetate
EDRF	=	Endothelium-derived Relaxing Factor
EGF	=	Epidermal Growth Factor
ELISA	=	Enzyme-linked Immunosorbent Assay
eNOS	=	Endothelial Nitric Oxide Synthase
EOC	=	Essence of Chicken
Fab	=	Fragment of Antigen Binding
FAD	=	Flavin Adenine Dinucleotide
Fc	=	Fragment Cystallizable
GEL	=	Gelatin
GLU	=	Glucosamine
GOT	=	Glutamic-oxalacetatic transaminase
GPT	=	Glutamic-pyruvic transaminase
$H^{\cdot}$	=	Hydrogen Atom
H_2O_2	=	Hydrogen Peroxide
HI	=	Hypertrophy Index
HRE	=	Haemoglobin Regeneration Efficiency
HRP	=	Horseradish Peroxide
IgA	=	Immunoglobulin A
IgD	=	Immunoglobulin D
IgE	=	Immunoglobulin E
IgG	=	Immunoglobulin G
IgM	=	Immunoglobulin M
iNOS	=	Inducible Nitric Oxide Synthase
IP	=	Intraperitoneal

LPO	=	Lipid Peroxidation
MAT	=	Mental Arithmetic Test
MDA	=	Malondialdehyde
MODS	=	Multiple Organ Dysfunction Syndrome
MWCO	=	Molecular Weight Cut Off
NIMGU	=	Non-insulin-mediated Glucose Uptake
nNOS	=	Neuronal Nitric Oxide Synthase
NO	=	Nitric Oxide
NO_2^-	=	Nitrite
NO_3^-	=	Nitrate
NOS	=	Nitric Oxide Synthase
NS	=	Normal Saline
$O_2^{\cdot -}$	=	Superoxide
$^{\cdot}OH$	=	Hydroxyl Free Radical
$ONOO^-$	=	Peroxynitrite Anion
OPD	=	o-phenylenediamine dihydrochloride
PAS	=	Pyrogallol Autooxidation System
PBS	=	Phosphate Buffered Saline
POMS	=	Profile of Mood State Questionnaire
$R^{\cdot}$	=	Free Radical
REE	=	Resting Energy Expenditure
$RO^{\cdot}$	=	Alkoxyl Radical
$ROO^{\cdot}$	=	Peroxyl Radical
ROS	=	Reactive Oxygen Species
RNO_x	=	Reactive Nitrogen Oxide Species

SBP	=	Systolic Blood Pressure
SC	=	Subcutaneous
S.E.M.	=	Standard Error of Mean
SHR	=	Spontaneously Hypertensive Rat
SHRSP	=	Stroke-prone Spontaneously Hypertensive Rat
SIgA	=	Secretory Immunoglobulin A
SMT	=	Short-term Memory Test
SOD	=	Superoxide Dismutase
TBA	=	Thiobarbituric Acid
TBARS	=	Thiobarbituric Acid Reactive Substances
TBS	=	Tris Buffered Saline
TBSA	=	Total Body Surface Area
TEP	=	1,1,3,3-tetraethoxypropane
TFG	=	Transforming Growth Factor
VH	=	Heavy Chain Variable Region
VL	=	Light Chain Variable Region
WKY	=	Wistar Kyoto Rat

CHAPTER 1

INTRODUCTION

1.1 Introduction

Chinese people, particularly those in Southeast Asian Chinese communities, have traditionally regarded essence of chicken (EOC) as a natural and nutritious remedy. EOC is believed to increase the body's resistance to illness by enhancing the immune response, removing some harmful stress-related factors from the body and maintaining body balance. Recent studies suggest that some components in EOC are capable of activating immune cells to scavenge pathological free radicals in cultured cells and an artificial auto-oxidation system, whilst suppressing hyperglycaemia in a rat model (Candlish, 1998; Yamano *et al.*, 2001). However, few studies have shown the in vivo effects of neat EOC on immunological responses and biochemical alterations, whether the body is in either a normal or a traumatized state.

The first chapter reviews basic knowledge on immunoglobulins, free radicals and blood glucose and background on EOC. The review of immunoglobulins includes molecular structure, class and subclass, and biological significance and function. For free radicals, this review will focus on the chemical and biological properties and synthetic pathways of nitric oxide (NO) and lipid peroxidation (LPO) as they relate to disease and detriments to health. Blood glucose is the body's major energy source and its concentration in serum must be carefully controlled to maintain proper cell

functions. Blood glucose level regulatory mechanisms will also be covered in this chapter.

1.2 Background to the Study

1.2.1 Essence of Chicken

Essence of chicken (EOC) was found in 1835 and is a well-known health food on the market. The reputation of EOC relies on its health benefits and its convenience to purchase and consume. EOC has become a remedy that caters to modern people's needs. During the past hundred years, EOC has spread widely in Southeast Asian Chinese societies, where it is consumed as a nutritious remedy.

Today, while keeping up with the busy pace of a fast-moving world, more and more people are becoming aware of their own health. This has increased demand for various kinds of health supplements and functional foods. In particular, EOC is taken by most people when they are recovering from an illness, and after pregnancy, surgery, etc.: critical times when more energy is needed to bolster the body's defences. EOC is also regarded as a natural health supplement that can be taken daily to improve mental fatigue and relieve stress.

EOC is a water extract made from high quality, healthy, certified and fresh chickens. During the extraction process, the chickens are treated by advanced double-boiling manufacturing processes in excess of 100^{o}C for several hours. The water extract is rich with nutrients that are harvested from the chicken. The meat and fat are removed

from the EOC and then the extract is concentrated and bottled to eventually become EOC, which can be easily purchased in the marketplace.

As EOC is the water extract of steamed whole chicken, EOC contains various kinds of nutrients from chicken meat, particularly dipeptides. Anserine and carnosine are the two most abundant dipeptides in EOC. According to the work from Tinbergen and Slump (1976), chicken meat contains higher anserine/carnosine ratio than other kinds of meat (the anserine/carnosine ratio for beef, pork and chicken meat are 0.06-0.2, 0.02-0.1 and 2.2-5.5 respectively). Another study from Huang and Kuo (2000) also reported that undemineralized poultry (chicken, duck and turkey) meat extracts contain large amount of carnosine, anserine, heme and non-heme iron. The nutrition facts and components of EOC are shown in Table 1-1 for reference.

Since the 1970s, the efficacy and benefits of EOC on human health have been confirmed consecutively through a series of scientific research studies. The first study was done by Geissler, Boroumand-Naini and Tomassen in 1989. The study included two separate sets of 20 healthy young Chinese college students (10 males and 10 females in each group). One group of subjects was provided with essence of chicken (70ml/bottle) and an identical appearance placebo which added small amount of caramel; while the other group of subjects was supplied with a more concentrated essence of chicken (70ml/bottle) and plain water as control. The activity and food intake of the subjects were manipulated one day before the experiment in order to standardize experimental conditions. The metabolic rate of each subject was measured by indirect calorimetry using Douglas bags for about 5 minutes at 15 minutes interval for 2 hours. The effects of EOC on thermic response were compared

with placebo and water by analysis of variance (ANOVA) and the $p<0.05$ was considered to have critical difference. The authors found that chicken essence was capable of elevating human thermic response by 8% to 12 % above baseline and the stimulation was dose-dependent.

Ikeda *et al.* further consolidated this finding in 2001. Such crossover design study investigated the thermogenic effects of chicken essence tablets on resting energy expenditure (REE). Eight male and nine female healthy college students were supplied with spray-dried chicken essence tablets or skim milk protein tablets (control). The treatments (chicken essence or control) were given at random order and there was one week between the two treatments. The temperature and noise of the laboratory was controlled. The subjects were told to take a 20 minutes rest before and after the experiment. The REE of each subject was measured before consuming the tablets and then every 30 minutes for 2 hours after consuming the tablets. The data was analysed with paired t-test and $p<0.05$ was considered to be statistically significant. The study revealed that the consumption of chicken essence tablets could increase REE in human subjects while control trials caused no effect.

Apart from the elevation of metabolic actions, another study by William and Schey (1993) suggested that EOC is able to speed up the restoration of serum iron to normal levels in regular blood donors. In this randomized, double-blind, placebo-controlled study, 84 regular blood donors were randomized to receive EOC or caramelized placebo. Blood samples were collected prior to the experiment. Each donor was asked to consume a bottle of fresh EOC or an indistinguishable placebo (both are 70ml) twice a day for 3 weeks. Then each donor was venesected 450ml of blood and

was requested to come back 4 days after donation and at weekly intervals for blood sample collections. The concentrations of haemoglobin, serum iron and ferritin were determined by Sysmex E500, ferrozine method without deproteinization and radio-immunoassay respectively. For data analysis, ANOVA was used and significant level was set at 5%. There were 24 donors on EOC and 23 donors on placebo who have finished the experiment. It is found that the female donors on EOC have faster recovery on serum iron at one week after blood donation, while placebo did not. Male donors on EOC also have such beneficial effect but the magnitude is smaller than female.

A parallel finding was reported by Geissler and colleagues in 1996. The study contains four experiments. The first experiment was a preliminary study to determine the effects of EOC on blood parameters of anaemic rats on an iron deficient diet. Thirty anaemic adult male Sprague-Dawley rats (haemoglobin level was about 9g/dL) were ranked by haemoglobin level and assigned to a treatment group or a control group alternately. The rats in treatment group and control group were provided with EOC and water respectively (free access) and diet with minimal adequate nutrients was supplied. Blood haemoglobin, haematocrit and serum iron levels were measured at approximately one week interval by cyanmethemoglobin method, haematocrit centrifuge and an iron separation from protein method respectively. The differences between groups were found out by ANOVA. In the second experiment, the procedures were same as first experiment except the starting haemoglobin level of the rats was at 6g/dL. The third experiment was aimed to determine the effects of lyophilized EOC in basic diet (lower iron availability) on haemoglobin, red blood cell count and haematocrit. Thirty anaemic female Sprague-Dawley rats (35-days-old)

were ranked in order of haemoglobin and divided into three groups as the first experiment. Basal diet with different iron sources were provided to rats in different groups, included reference diet ($FeSO_4$ as iron source), control diet (rice bran as iron source) and EOC diet (1% EOC added to control). After feeding for one week, the animals were sacrificed and blood samples were collected. The blood parameters that mentioned above were analyzed by an automatic haemato-analyser and the haemoglobin regeneration efficiency (HRE) was calculated. The forth experiment was designed to find out the effects of different level of lyophilized EOC added to the diet with limited iron on HRE. Twenty-five anaemic rats were divided into three groups according to their haemoglobin level. The rats in different groups were fed with control diet (same as the third experiment), control diet with 0.2% lyophilized EOC and 1% lyophilized EOC respectively. The animals were sacrificed and blood samples were collected to measure the blood parameters (same as the third experiment after one week feeding). HRE was also calculated. According to these experiments, the authors found that EOC can significantly increase the levels of haemoglobin when compared with control group (fed on water) ($p=0.01$). EOC can also partially restore the HRE compared with the other groups. They Concluded that EOC stimulated haemoglobin restoration in iron-deficient rats by enhancing the bio-availability of food iron.

Apart from physical health, EOC has been found to be useful in promoting recovery from mental fatigue caused by workload in humans (Nagai *et al.*, 1996). This crossover design study has recruited 20 male university students. They were divided into two groups according to a pre-arithmetic calculation test which aimed to equalize the abilities in both groups. Then the subjects of one group were asked to consume

two bottles of EOC (70ml/bottle) in the morning for 7 consecutive days. After a three weeks wash out period, the subjects were provided with a placebo with same appearance with EOC (a 7.2% gelatine and 0.3% caramel solution) for another 7 days. The subjects were required to receive two types of mental workload tests, including a mental arithmetic test (MAT) and a short-term memory test (SMT), on the final day of consuming each solution. Blood samples were collected before and after the tests. The profile of mood state questionnaire (POMS) was used to analyze the subjective feeling of the subjects during the tests. The serum cortisol concentration was determined by immunochemiluminescence. The subjects of the other group received the treatments that similar to the previous group, except the order of receiving EOC and placebo was reversed. A two-factors ANOVA was used to determine the treatments that have statistically significant. This study revealed that the subjects on EOC have a faster recovery of mean cortisol level than the placebo group. The result of POMS questionnaire also showed that the EOC group has more stamina but less fatigue than the placebo group. This benefit is ascribed to EOC's ability to enhance the metabolism of stress-related substances in blood.

Azhar, Abdul Razak and Mohsin (2001) have also demonstrated that the consumption of EOC assists the recovery status of anxious subjects during psychotherapy. Twenty-two male and female patients with anxiety were divided into three groups randomly. All patients were provided with placebo (P) or active compound (A) (pure chicken extract) in different order according to their grouping. For the patients in first group, they were supplied in A-P-A model for three months (one month each). Psychological and physical tests were given to each subject at the end of every month. Similarly, the model for the second was A-A-P and the third group was P-A-P. Chi-

square or t-test was used to analyze the data. The results showed that there were improvements in psychological tests (Beck Anxiety Inventory and Hamilton Anxiety Scale) and physical states (pulse rate and systolic blood pressure (SBP)) after the consumption of EOC.

Clinically, prior studies have shown that EOC may be useful as a treatment for hypertension and related tissue injury, as it has demonstrated anti-hypertensive effects in a rat model (Matsumura *et al.*, 2001; Matsumura *et al.*, 2002). The study conducted by Matsumura and colleagues (2001) was used hypertensive animal model, induced by deoxycorticosterone acetate (DOCA) and salt. Right kidney of 17 male Sprague-Dawley rats (6-week-old) was removed by operation. After one week recovery period, they were divided into a sham-operated group (n=6) and a group with DOCA-salt-induced hypertension. The DOCA-salt group was further divided into a normal diet group (n=6) and an EOC-containing diet group (n=5). EOC was given in a powder form and mixed with normal commercial rat chow at 0.1 w/w%. The authors claimed that the feeding of this diet is approximately equal to half to one bottle of EOC/human/day. In the five-week experiment, SBP was measured weekly, urine was collected overnight for urinary protein analysis, and the heart, left kidney and aorta were excised for morphometric analysis. ANOVA analysis revealed that the consumption of EOC is capable to the development of hypertension induced by DOCA and salt. The hypertension-related diseases, cardiovascular hypertrophy, renal tissue injury and increase of urinary protein excretion were also relieved by EOC consumption. The authors suggested that EOC may be useful as a prophylactic treatment for hypertension and related tissue injury. A similar study was conducted using 10 male stroke-prone spontaneously hypertensive rats (SHRSPs) (6-week-old)

(Matsumura *et al*., 2002). The rats were divided into an EOC-fed group (n=5) and a control group (n=5). EOC was given as powder and was added to the drinking water at concentration of 0.3-0.8mg/ml, while the rats in control group were provided with plain water. The rats were allowed to access the water freely. The dosage of EOC is approximately equivalent to 100mg of EOC powder/kg/day (one bottle of EOC/human/day). After 19-week experiment, the parameters same as the previous study plus blood creatinine level were examined. It is found that EOC feeding can significantly prevent the development of hypertension and protect renal functions by suppressing the elevation of blood urea nitrogen (BUN) and plasma creatinine levels in SHRSPs. The findings are parallel with the study that done by the same authors in 2001.

Sim (2001) also reports that EOC has anti-cardiac hypertrophic and anti-arteriosclerotic actions in animal models. This study investigated four effects of EOC on hypertensive rat model. The first experiment was to investigate the effects of EOC on the blood pressure of spontaneously hypertensive rats (SHR) and their control Wistar Kyoto rats (WKY). Six SHR and six WYK were implanted with a telemetry blood pressure transmitter for measuring blood pressure and heart rate. The parameters were measured for 15 days. Then 0.25ml EOC/100g body weight was given to both SHR and WKY for 30 days consecutively. Blood pressure and heart rate were monitored during the feeding period and for 15 thereafter. The result showed that the intake of EOC did not affect the blood pressure and heart rate of both normal and hypertensive rats ($p>0.05$, ANOVA). The second experiment investigated the effects of EOC on the development of blood pressure in the SHR for 90 days. Two groups of six SHR were fed with EOC at 0.25 ml/100g body weight and saline

as placebo for 90 days daily. Then each rat was implanted with a telemetry blood pressure transmitter for measuring of blood pressure for 15 days after the treatments. The results showed that the prolonged intake of EOC caused no effect on the development of hypertension in the SHR. The third experiment found the effects of EOC on Sprague-Dawley rat model with induced cardiac hypertrophy. Eight groups of six Sprague-Dawley rats were induced with hypertrophy by operation. The rats in each group were fed with EOC for 4 days at 0.01, 0.02, 0.04, 0.08, 0.16 and 0.32ml/100g body weight according to the group allocation. The rats in a group were fed with EOC at 0.4ml/100g body weight and co-administrated with indomethacin (this compound is added to show that the effects of EOC on anti-cardiac hypertrophy are merely due to the anticipation of des-aspartate-angiotensin I or not). The rats in control group were fed with saline and a sham-operated group was used as reference. The carotid and femoral blood pressures of the animals were measured by pressure transducers on day 4 of the experiment and the extent of coarctation was indicated by the difference between the two readings. Hypertrophy index (HI) was measured by the ventricle weight (mg) over the body weight of the animal (g). The results showed that EOC can attenuate the acute experimentally-induced cardiac hypertrophy in dose-dependently manner, and the effects may cause by more than one compound in EOC. A parallel experiment was done to investigate whether laboratory-prepared chicken-meat extracts and pork extracts have anti-cardiac hypertrophy effects or not. The chicken meat and pork (600g each) were boiled in 1.2L purified water for 7 hours separately. Then the mixtures were centrifuged and filtered to obtain the clear fat-free supernatant. The chicken-meat and pork extracts were fed to four groups of Sprague-Dawley rats at concentrations 0.2, 0.4, 0.8 and 1.6ml/kg body weight per day orally (each group contains 6 rats and a control group was fed with normal saline). The

results appeared that the effects are only presence in the chicken-meat extract but not the pork extract. The difference in efficacy may be due to the different anserine and carnosine ratio among pork and chicken meat. At the last experiment, the effects of EOC on age-related development of hypertension, cardiac hypertrophy and arteriosclerosis in the SHR and WKY were investigated. One-month-old SHR were divided into three groups which the rats in each group were fed with water (control), 2.5% (v/v) EOC and 10% EOC; while the normotensive control WKY were divided into two groups and the rats were fed with water (control) and 10% EOC. All of the rats were allowed to access rat chow and either water or EOC freely for 11 months. The second experiment was used 5-moth-old SHR. The rats were fed with 10% EOC for 7 months. All groups in this experiment contain 12 rats. At the end of 11 months, HI of the animal was determined as the third experiment. A section of the abdominal aorta was removed and fixed to measure the thickness of aortic smooth muscle layer by an image analysis system. It was found that chronic feeding of EOC is capable to attenuate the age-related development of cardiac hypertrophy in dose-independent manner, and have a significant anti-arteriosclerotic effect.

For other beneficial effects of EOC on human health, Xu and Sim's (1997) study noted that EOC may cause a rise in the level of 5-hydroxyindole acetic acid (5-HIAA) in the cerebrospinal fluid (CSF) in a rat model, which is linked to the elevation of 5-hydrotryptamine (5-HT) activity in the brain and results in activation of related physiological processes like sleep improvement, mood elevation, analgesia, facilitation of motor output and regulation of circadian rhythm. The experiment included 12 three-month-old Sprague-Dawley rats in a control group and an experimental group. The rats in the control group and the experimental group were

fed with 1ml of saline and fresh undiluted EOC for 3 consecutive days respectively. A dialysing probe was implanted to measure the 5-HIAA in the CSF. The results were compared using ANOVA. The findings showed that the oral administration of EOC for 3 days caused significant increase of 5-HIAA in rats ($P<0.0005$), which means EOC is capable of rising the 5-HT activity of the animals.

Tsi *et al.* (2003) investigated the effect of EOC with glucosamine (GLU) and showed significant anti-arthritic effects in a collagen-induced arthritis rat model. This study comprised of one control and four experimental groups and each group has 10 female dark agouti (DA) rats (11-week-old). Collagen-induced arthritis (CIA) was induced to the rats by injection of 300μg bovine type-II collagen in four to six sites on the dorsal skin on day 0 of the experiment. Then the rats in the control group were fed with basic food. The rats in the three experimental groups were fed with 1.2% GLU admixed with basic food, 0.8% EOC admixed with basic food and a combination of 1.2% GLU + 0.8% EOC admixed with basic food for 25 consecutive days. Then the volumes of the foot pads on the right and left hind limbs were measured on days 10, 12, 13, 14, 15, 17, 20 and 25 by plethysmometer. The foot pads were isolated for histopathological analysis on day 25. The area surrounding the 5 metatarsal bones were evaluated and scored based on the degree of periosteal new bone formation, periostitis, inflammatory cell infiltration and edema. The differences of the mean values between control group and experimental groups were analyzed by one-way ANOVA and the significant level was set at $p<0.05$ and $P<0.01$. The findings showed that EOC plus GLU can attenuate the severity of edema and inflammation caused by CIA effectively in animal model.

The latest study on EOC reports that it can increase the colostrum levels of lactoferrin, epidermal growth factor (EGF) and transforming growth factor (TGF)-β2 in postpartum women (Chao *et al.*, 2004). The experiment involved a control group and an experimental group, each contained 15 healthy pregnant women with 23-39 years old from a Taiwan hospital. The subjects in the experimental groups were given one bottle of EOC (70ml) three times a day within 30 minutes after each meal. The period was last from the 37^{th} week pregnancy to 3 days postpartum. During the experimental period, blood sample was collected daily and milk sample was collected twice a day in the morning and afternoon. The levels of total cholesterol and triacylglycerols, iron and unsaturated iron binding capacity in plasma; and the levels of casein, lactalbumin, lactoferrin in milk were measured. The total protein level, EGF, TGF-β2 and SIgA concentrations were analyzed in both plasma and milk. All data were analyzed by t-test and chi-square and the significant level was set at $p<0.05$. The findings showed that the plasma EGF level increased significantly by 236% in the women of EOC group. On the other hand, the levels of lactoferrin, EGF, and TGF- β2 in colostrums elevated significantly by 34%, 62% and 196% respectively. The study suggested that the consumption of EOC during gestation may benefit the growth and immune functions of infants through lactation.

Table 1-1. Compositions of Brand's Essence of Chicken

Ingredient	Amount		Source
Protein & Peptide			
Protein & Peptide	83.0	mg/ml	Geissler *et al.*, 1996
Amino Acid (free)	3.1	mg/ml	Geissler *et al.*, 1996
Hexose	0.8	mg/ml	Geissler *et al.*, 1996
Lipid	0.4	mg/ml	Geissler *et al.*, 1996
Carnosine	5.0	mmol/l	Candlish, 1998
(β-alanylhistidine)	10.0	mg/g	Matsumura *et al.*, 2002 (in powder form)
Anserine (β-alanyl-1-	10.0	mmol/l	Candlish, 1998
methylhistidine)	30.0	mg/g	Matsumura *et al.*, 2002 (in powder form)
Mineral			
Calcium	26.0	µg/ml	Geissler *et al.*, 1996
Iron	1.0	µg/ml	Geissler *et al.*, 1996
Magnesium	32.0	µg/ml	Geissler *et al.*, 1996
Zinc	2.0	µg/ml	Geissler *et al.*, 1996
Potassium	1740.0	µg/ml	Geissler *et al.*, 1996
Sodium	550.0	µg/ml	Geissler *et al.*, 1996
Chlorine	1340.0	µg/ml	Geissler *et al.*, 1996
Phosphorus	480.0	µg/ml	Geissler *et al.*, 1996
Copper	2.0	µg/ml	Geissler *et al.*, 1996
Manganese	5.0	µg/ml	Geissler *et al.*, 1996
Sulphur	500.0	µg/ml	Geissler *et al.*, 1996
Selenium	0.05	µg/ml	Geissler *et al.*, 1996
Vitamin			
Vitamin A	0	µg/100ml	Nutrition Values provided by the Company
Vitamin B_1	<0.01	mg/100ml	Nutrition Values provided by the Company
Vitamin B_2	1.0	mg/kg	Geissler *et al.*, 1996
Vitamin B_6	0.37	mg/kg	Geissler *et al.*, 1996
Vitamin B_{12}	0.002	mg/kg	Geissler *et al.*, 1996
Niacin	6.4	mg/kg	Geissler *et al.*, 1996
Folic Acid	0.15	mg/kg	Geissler *et al.*, 1996
Vitamin C	15.0	mg/kg	Geissler *et al.*, 1996

Table 1-1. (Cont'd) Composition of Brand's Essence of Chicken

Ingredient	Amount		Source
Others			
Calories	38.0	kcal/100ml	Nutrition Values provided by the Company
Calories from Fat	0	kcal/100ml	Nutrition Values provided by the Company
Total Fat	0	g/100ml	Nutrition Values provided by the Company
Saturated Fat	0	g/100ml	Nutrition Values provided by the Company
Cholesterol	0	g/100ml	Nutrition Values provided by the Company
Carbohydrate	1.3	g/100ml	Nutrition Values provided by the Company
Fibre	0	g/100ml	Nutrition Values provided by the Company

1.2.2 Immunoglobulin

Immunoglobulins are antibodies that belong to a group of glycoproteins and are secreted by B-lymphocytes. They are the first molecules that participate in specific immune recognition. When invading antigens are encountered, B-lymphocytes proliferate and a huge amount of antibody is manufactured. Immunoglobulins play an important role in the humoral immune response, which includes recognizing foreign antigens and provoking immune cascades (Janeway *et al.*, 1999).

Up to now, five major classes of immunoglobulin have been classified. They are termed immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin E (IgE) and immunoglobulin D (IgD). Each class has its own molecular size, charge, amino acid sequence and carbohydrate content. Within the classes, some may contain subclasses as well. As an example, IgG contains four subclasses: IgG_1, IgG_2, IgG_3 and IgG_4, which all have different biological functions.

The essential structure of all immunoglobulin molecules is a pair of light chains linked with a pair of heavy chains by covalent force, disulphide bonds and non-covalent force. The class of immunoglobulin is decided by the type of heavy chain that the molecule possesses (Table 1-2); while the type of light chain may be either κ or λ. There is no functional difference between the two types of chain, and either type of light chain may be found in any type of the five major classes. However, the ratio of the two types of light chain varies from species to species.

Table 1-2. Corresponding classes of immunoglobulin with different types of heavy chain

	Immunoglobulin								
	IgG_1	IgG_2	IgG_3	IgG_4	IgM	IgA_1	IgA_2	IgD	IgE
Type of Heavy Chain	γ_1	γ_2	γ_3	γ_4	μ	α_1	α_2	δ	ε

Two pairs of light and heavy chains are arranged in a Y shape. Disulfide bonds link the two heavy chains to each other and each heavy chain is linked to a light chain by a disulfide bond. The two heavy chains and the two light chains are identical for any immunoglobulin molecules in the same class. Figure 1-1 illustrates the locations of the heavy and light chains, the bonds that bind them together and the antigen-binding regions.

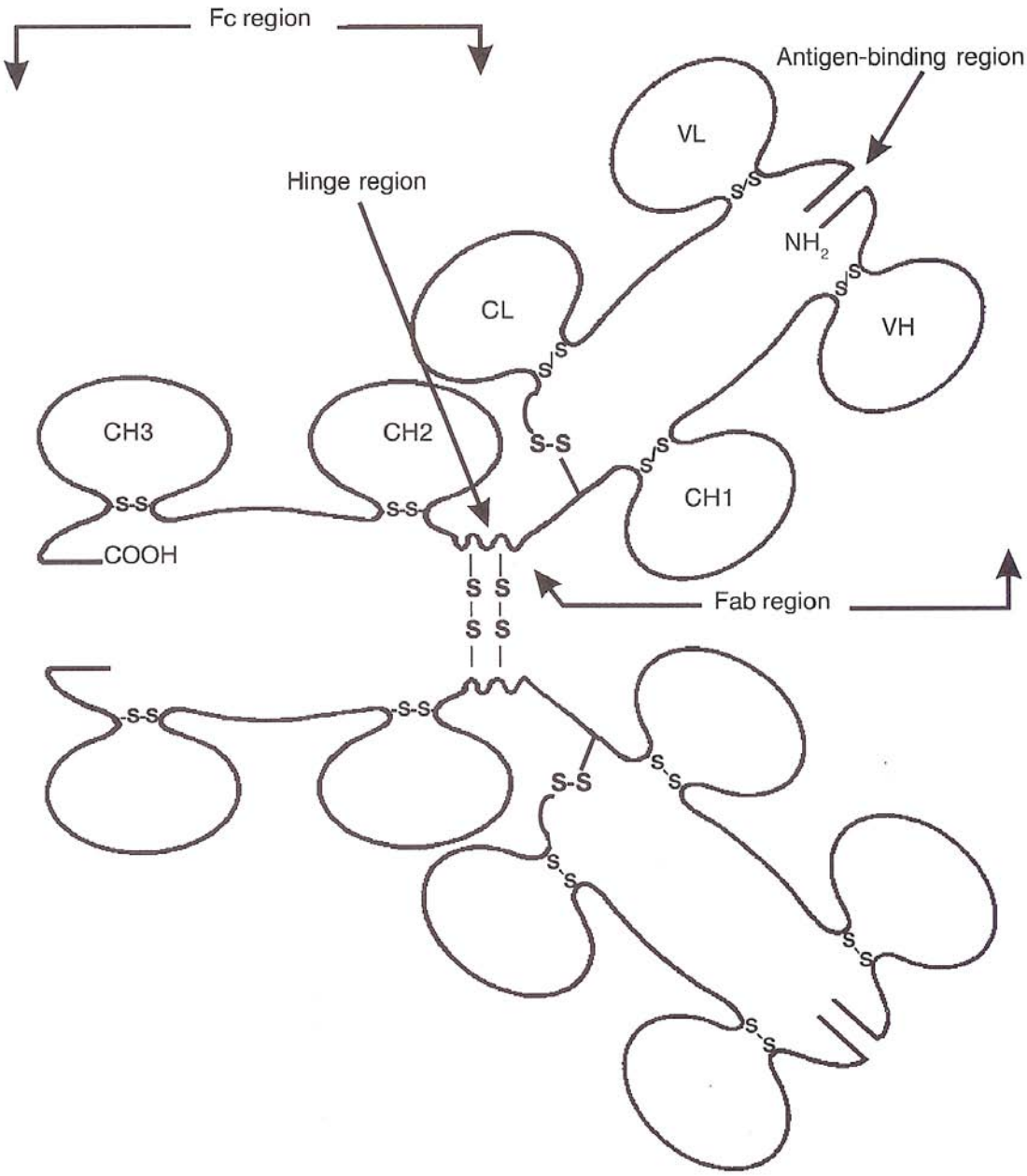

Figure 1-1. The typical structure of an immunoglobulin molecule (from Eales, 1997, p.41)

The ends of both heavy and light chains are amino-terminals, which are a single variable region or domain (VL and VH). Because it forms the antigen-binding site that attaches to the surface of antigens, the variable region is the most important part of the immunoglobulin structure. For the region of the heavy chain that has not bonded to light or heavy chains, constant regions (CH) are formed. The number of these regions in an antibody molecule varies with the type of immunoglobulin, with three in IgG, IgA and IgD and four in IgM and IgE. Light chains have only one constant region (CL). There is little alternation in these regions of the secondary and tertiary structure of the proteins, which are thus called constant.

Antibody molecules can be divided into two functional domains. The first is a fragment of antigen binding (Fab), which consists of the two VH-CH1 domains and two VL-CL domains; the second is fragment crystallizable (Fc), which consists of the CH2 and CH3 domains (and CH4 in IgM and IgE). The region where the Fab and Fc join together is called a hinge region. This region consists of a high number of proline and cysteine residues. Cysteine can form interchain disulphide bonds that are used to keep the shape of the molecule intact.

Each type of immunoglobulin has different biological functions in the immunological response. There functions are summarized in Table 1-3.

Table 1-3. The major characteristics of the five classes of immunoglobulin

Type of Immunoglobulin	**Percentage in the Circulating System**	**Subclass (es)**	**Major Characteristics**
IgG	70 – 75%	IgG_1 (70%) IgG_2 (20%) IgG_3 (8%) IgG_4 (2%)	- Produced during secondary immune response - The only antibody that can neutralize toxin - Mainly binds to and opsonizes viruses and bacteria - Only antibody that can be transported through the placenta
IgA	15 – 20%	IgA_1 (mainly present in serum) IgA_2 (mainly present in mucous secretions)	- Major antibody secreted at mucous membranes - Present in various body fluids - Can form dimers, trimers, tetramers and pentamers - Forms secretory IgA (sIgA), which exists in external secretions - Functions by blocking the access of the antigen instead of destroy it
IgM	10%		- Produced in primary response - Confined to peripheral circulation - Can form pentamers with 10 antigen-binding sites - Most efficient in initiating complement cascade
IgE	Extremely low concentration		- Bound to the surface of mast cells and basophils - Mainly involved in allergic reaction and against parasitic infections - Enhances vascular permeability to allow the migration of leucocytes at the site of infection
IgD	< 1%		- Expressed on the surface membrane of B cells with IgM - Exact biological role is still unknown: may be involved in the development of B-lymphocytes - Most sensitive to proteolytic cleavage and high temperature

1.2.3 Free Radicals

Free radicals are defined as molecule that can exist independently and contain at least one unpaired electron in an orbital. Due to presence of the singlet electron, free radicals are highly reactive, unstable and have a short half-life. They always seek to (1) donate the singlet electron to another molecule, or (2) take an electron from another molecule, or (3) join onto another molecule to form a covalent bond (sharing of electron). All possible actions aim to stabilize the excess electron. However, the action of "taking" or "adding" an electron to a stable molecule may result in the potential initiation of chain reactions or the oxidation of other molecules. Some highly reactive oxygen containing free radicals are called reactive oxygen species (ROS). They are generated in various oxidative metabolisms. These high energy ROS are potentially cytotoxic to the cell as they can react with lipids, proteins and DNA in cells, as a result they may disrupt normal cellular processes and cause cellular damage: such a process is called oxidative stress. Typical ROS include hydrogen peroxide (H_2O_2), superoxide radicals ($O_2^{\cdot -}$) and hydroxyl free radicals ($^{\cdot}OH$).

Free radicals are not strangers to the human body. Day by day, they are generated as by-products during normal metabolic processes inside cells. One of the typical examples is superoxide, which is released "accidentally" during the reduction of oxygen to water by mitochondrial electron transport. The other example is leucocytes (including macrophages and neutrophils): these immune cells can liberate free radicals to kill extracellular bacteria (Punchard and Kelly, 1996). However, these self-generating free radicals are toxin-free to us as our bodies have effective antioxidant defence systems, such as enzymes that can inactivate peroxides, proteins to sequester transition metals and compounds that can scavenge free radicals (e.g.,

superoxide dismutases (SOD) and catalases) (De Zwart *et al.*, 1999), which can remove or alter the free radicals to non-toxic substances continuously before they can accumulate to harmful levels. The human body also has a repair system to remove oxidatively damaged molecules (Halliwell, Gutteridge and Cross, 1992). However, when free radicals are produced in excess amounts in particular situations, or when the antioxidant and repair systems of the body are impaired, free radicals cannot be removed effectively and excess free radicals are capable of increasing the oxidative stress of cells, which means they attack or "poison" the cells by oxidizing them.

1.2.3.1 Nitric Oxide

Nitric oxide (NO) is a short-lived diatomic free radical. It has been found to be an endothelium-derived relaxing factor (EDRF) in the body and to be involved in major regulatory functions of the body, ranging from altering the cardiovascular system to modulating neuronal function (Wink and Mitchell, 1998). Some studies have also shown that NO has vital functions in vasodilation and hyperpolarization in endothelial cells (Moncada and Higgs, 1991; Vanhoutte, 2004). In fact, NO has paradoxical roles in many biological reactions within the body. Normally, a low level of NO is needed for protecting the cell functions, promoting vasodilation in the peripheral circulation system and preventing the adherence of platelets and neutrophils in the microvasculature (Horton, 2003). However, when high levels of NO are present, they may contribute to some detrimental effects, which will be discussed in a later chapter.

NO is produced from the terminal guanidino nitrogen atom of arginine in the presence of nitric oxide synthases (NOS). NOS exist in three different isoforms: endothelial NOS (eNOS) and neuronal NOS (nNOS) belong to constitute isoforms, while the

third isoform is inducible NOS (iNOS) (Witte and Barbul, 2002). The constitute NO synthases are permanently active in generating low concentrations of NO, which manifest regulatory effects on vascular tone and permeability, cellular adhesion, neurotransmission, bronchodilation, systemic blood pressure, inhibiting platelet aggregation and renal function (Wink and Mitchell, 1998). Conversely, iNOS are activated by a variety of growth factors, inflammatory stimuli and traumatic injury to generate high concentrations of NO. In concert with the impairment of the antioxidant and repair systems, iNOS releases the mainstay of NO, which is responsible for impairing cell functions and causing free radical-mediated diseases.

NO is a relatively less reactive free radical than others, such as superoxide. However, NO can react with superoxide, oxygen, and thiol groups (-SH) to yield various types of powerfully reactive nitrogen oxide species (RNO_x) products, such as nitrosothiols, peroxynitrite anion ($ONOO^-$) and nitrites. Within RNO_x, peroxynitrite is formed by NO and superoxide when they are at near equimolar ratio in biological systems. It is not a free radical but readily to react with a variety of biomolecules, such as proteins, lipids and DNA (Szabó, 2003; Virág *et al.*, 2003). Peroxynitrite can react with unsaturated fatty acid-containing liposomes, which results in the initiation of lipid peroxidation. Moreover, exposure of low-density lipoprotein to peroxynitrite results in the oxidation of unsaturated fatty acids (Graham *et al.*, 1993). The mechanism of initiation of lipid peroxidation by peroxynitrite is still unclear. It may involve either abstraction of a bis-allylic hydrogen by the “the hydroxyl radical-like” activity peroxynitrite, or induced homolysis of peroxynitrite by the unsaturated fatty acid. (Hogg and Kalyanaraman, 1999). Undoubtedly, peroynitrite plays important roles in most NO-mediated diseases and tissue damage. Apart from initiation of lipid

peroxidation, studies revealed that peroxynitrite involves in direct inhibition of mitochondrial respiratory chain enzymes, inactivation of glyceraldehydes-3-phosphate dehydrogenase, inhibition of member Na^+/K^+ ATP-ase activity, inactivation of membrane sodium channels, oxidative protein modifications with cytotoxic effects, and also trigger cell apoptotic death and necrosis, DNA strand breakage and damage the enzymes that are involved in DNA repair (Szabó, 2003; Virág *et al.*, 2003). RNO_x readily reacts with transition metals (such as zinc and iron in haem-containing proteins) and thiol groups in enzymes and proteins, which may lead to inhibition of enzymes and cause depression of ATP production and cell death by producing toxic cyanide (CN^-) and carbon monoxide (CO). NO can also change the conformation of enzyme structures and result in the inhibition of oxygen binding to haemoglobin (Brenann, Thomas and Langdon, 2003).

Nitrite (NO_2^-) and nitrate (NO_3^-) are the stable catabolic products of NO. When NO enters a biological system, it undergoes a number of reactions as shown below. NO_2^- and NO_3^- are usually used as markers for the determination of NO levels in bodily fluids. However, as the relative proportions of NO_2^- and NO_3^- are variable, their total (NO_x) is used to indicate the NO level.

$$NO + O_2^- \longrightarrow ONO_2^- \xrightarrow{H^+} NO_3^- + H^+$$

$$2NO + O_2 \longrightarrow N_2O_4 \xrightarrow{H_2O} NO_2^- + NO_3^-$$

$$NO + NO_2 \longrightarrow N_2O_3 \xrightarrow{H_2O} 2NO_2^-$$

1.2.3.2 Lipid Peroxidation

Lipid peroxidation (LPO) is defined as the oxidative deterioration of polyunsaturated lipids, which means lipids containing any number of carbon-carbon double covalent bonds. Rancidity is the oxygen-dependent deterioration of lipids. This has been noticed since ancient times as a problem in the storage of fats and oils. When they are oxidized, their characteristics change in texture, colour, taste and odour (Gutteridge, 1995). The problems of rancidity can also extend to the storage of many domestic and industrial products nowadays, such as "polyunsaturated" margarines and cooking oils, paints, plastics, lacquers, and rubber, all of which can undergo oxidative damage (Halliwell and Gutteridge, 1989).

Prior to knowing the etiology of the detriments of LPO to organisms, we have to understand the structure of biological membranes. Cells and cell organelles are surrounded by membranes that are constructed of a lipid bilayer, whilst some proteins are loosely attached to the surface of the membrane. Because lipids are a main constituent of the membranes, the membranes contain large amounts of polyunsaturated fatty-acid side chains, which provide excellent sites for the occurrence of LPO. Therefore, LPO can damage the membrane as well as the proteins.

Like all chain reactions, LPO attacks the polyunsaturated fatty acid in three stages – initiation, propagation and termination. The first-chain initiation of a peroxidation is started by any species ($R^{\cdot}$) with sufficient reactivity to abstract a hydrogen atom ($H^{\cdot}$) from a methylene ($-CH_2-$) group. The attack results in the formation of a double bond in the fatty acid, which can weaken the C-H bonds nearby. Hence, the other C-H

bonds are more compromised to H˙ removal, whilst the polyunsaturated fatty acid chains of the membrane lipids are more sensitive to LPO. Some species that can initiate the LPO include hydroxyl free radical (˙OH), alkoxyl radical (RO˙) and peroxyl radical (ROO˙). After the removal of H˙, the molecule is prone to stabilize itself by rearranging and forming a conjugated diene. The conjugated diene can react with oxygen to give a peroxyl radical (ROO˙). Because it is capable of abstracting H˙ from another lipid molecule, ROO˙ has a vital role in LPO. These continuing and repeating reactions are important to the propagation stage of LPO. On the other hand, the peroxyl radicals may bind with H˙ to form lipid peroxides. The LPO reactions continue until all substrates are used up, unless the propagating reaction is terminated by a chain-breaking antioxidant such as vitamin E (Gutteridge, 1995).

There are two types of biological LPO. Nonenzymic LPO occurs as described before, when any free radical (R˙) with adequate energy removes an H˙ from a methylene carbon of an unsaturated fatty acid, while enzymic LPO only represents the generation of lipid peroxides at the active centre of an enzyme (Gutteridge, 1995). Both types of LPO are capable of generating powerful free radicals during the processes.

Malondialdehyde (MDA) is one of the lipid peroxides that is produced in LPO. In vivo environment, it is formed in large amounts during the LPO of liver microsomal fractions and other cell membrane fractions. MDA can attack amino groups on protein molecules to form both intramolecular cross-links and cross-links between different protein molecules. As MDA can react with 2-thiobarbitric acid (TBA) to

yield a coloured product, it is usually used as a bio-marker to measure LPO levels in blood or tissues (Halliwell and Gutteridge, 1989).

1.2.4 Diseases Related to Alterations of Nitric Oxide and Lipid Peroxidation Levels

The alteration of physiological NO levels in the body appears to be associated with various diseases and ageing processes. There is no doubt that tissue damage such as burn trauma can cause over-expression of NO synthases and exceed the normal plasma NO levels. There is an enormous body of literature providing evidence that abnormal NO production has a close relationship with a variety of oral diseases (Brennan *et al.*, 2003); a range of neurodegenerative disorders like Alzheimer's disease and Parkinson's disease (Emerit, Edeas and Bricaire, 2004; Fernández-Vizarra *et al.*, 2004; Jack C. de la Torre and Stefano, 2000; Knott, Stern and Wilkin, 2000; Lüth, Münch and Arendt, 2002; Riobó *et al.*, 2002); cardiovascular diseases like atherosclerosis, intimal hyperplasia and aneurismal disease (Barbato and Tzeng, 2004; Easki *et al.*, 2000; Handa *et al.*, 2004; Yokoyama, 2004); multiple sclerosis (Danilov *et al.*, 2003; Hill *et al.*, 2004; Nazliel *et al.*, 2002; Smith and Lassmann, 2002); tissue destructive diseases like arthritis (Abramson *et al.*, 2001; Borderie *et al.*, 1999; Chan and Mattiacci, 2001); and the ageing of a variety of tissues and organs (Kasapoglu and Özben, 2001; McCann *et al.*, 1998).

Similar to NO, an increase in LPO levels can provoke the occurrence of various diseases, particularly age-related ones. Several studies have confirmed the pathological roles of LPO in neurodegenerative diseases (Arlt *et al.*, 2002; Montine *et al.*, 2004; Ou, Zhang and Montine, 2002), atherosclerosis (Fabbi *et al.*, 2003; Miquel

et al., 1998), indirect causes of type 2 diabetes mellitus (Oranje *et al.*, 1998), inducing cancer by damaging DNA (Marnett, 2002), and ageing (Kasapoglu and Özben, 2001; Spiteller, 2001).

Apart from these detriments, increased NO levels after thermal injury have been found to be closely associated with the incidence of sepsis, multiple organ dysfunction syndrome (MODS), inflammatory response and immune dysfunction in severely burned patients (Horton, 2003; Manuel do Rosário Caneira da Silva *et al.*, 1998; Parks *et al.*, 1998; Rawlingson, 2003; Schwacha, 2003). In addition to the deleterious effects of NO, an elevation of LPO levels postburn also participates in the deactivation of tissue free radicals scavenging catalase and the development of burn shock and distant organ injury (Horton, 2003; Kumar *et al.*, 1995). There is no question that the development of such free radical-mediated defects postburn can promote the complications of burn injury, jeopardize recovery, increase susceptibility to infection and ultimately result in an increased morbidity and mortality rate after burn injury.

1.2.5 Blood Glucose

Blood glucose is the essential source of energy for the body, and is transferred through the bloodstream. Despite the typical intake of a variety of foods each day, the three main components of food are proteins, fats and carbohydrates. Blood glucose comes from these components, mostly from carbohydrates. After the foods are ingested, they are digested and broken down by the mechanical and enzymatic processes of the digestive system. These processes transform complex molecules into the form of simple and absorbable glucose, which is absorbed by the body.

As the “fuel of body”, the concentration of blood glucose must be carefully regulated in the bloodstream to ensure that all body tissues, especially the brain and central nervous system (CNS), have a stable and sufficient energy supply, whilst carrying out their functions properly. Blood glucose is regulated by a counteracting hormonal control system. In order to maintain the blood glucose concentration within a narrow range, this hormonal system works in an antagonistic manner – which means that at least two hormones work with opposite functions to balance the whole system. The two endocrine glands that secrete hormones to regulate the blood glucose system are the pancreas and the adrenals. The mechanisms of regulation on blood glucose level by pituitary gland works in a counterbalance manner: the anterior pituitary releases adrenocorticotropic hormone (ACTH) which stimulates the adrenal cortex to secrete cortisol. Cortisol is a “stress hormone” which is one of the essential hormones for life. It promotes gluconeogenesis, glycogen synthesis and glucose output in liver and depresses glucose uptake by muscle, adipose and other tissues. Consequently, blood glucose level increases. On the other hand, the rise of blood glucose may stimulate the secretion of insulin from pancreas which counterbalances the increase of blood glucose level (Figure 1-2).

The Islets of Langerhans is a special group of cells that is responsible for the secretion of insulin. Insulin is a hormone that can promote the conversion of blood glucose to its storage form, glycogen, to be stored in the liver (glycogenesis). Such conversion can down-regulate the blood glucose level when it is high and prevent the development of hyperglycaemia. Glucagon (from the pancreas) and epinephrine (from the adrenal glands) on the other hand possess the opposite function to insulin,

promoting the breakdown of glycogen in the liver to blood glucose (glycogenolysis). Glucagon and epinephrine work when the blood glucose level is low (hypoglycemia), and guarantee an adequate glucose supply to the organs and tissues. The regulation of blood glucose level by pancreas has been illustrated in Figure 1-3.

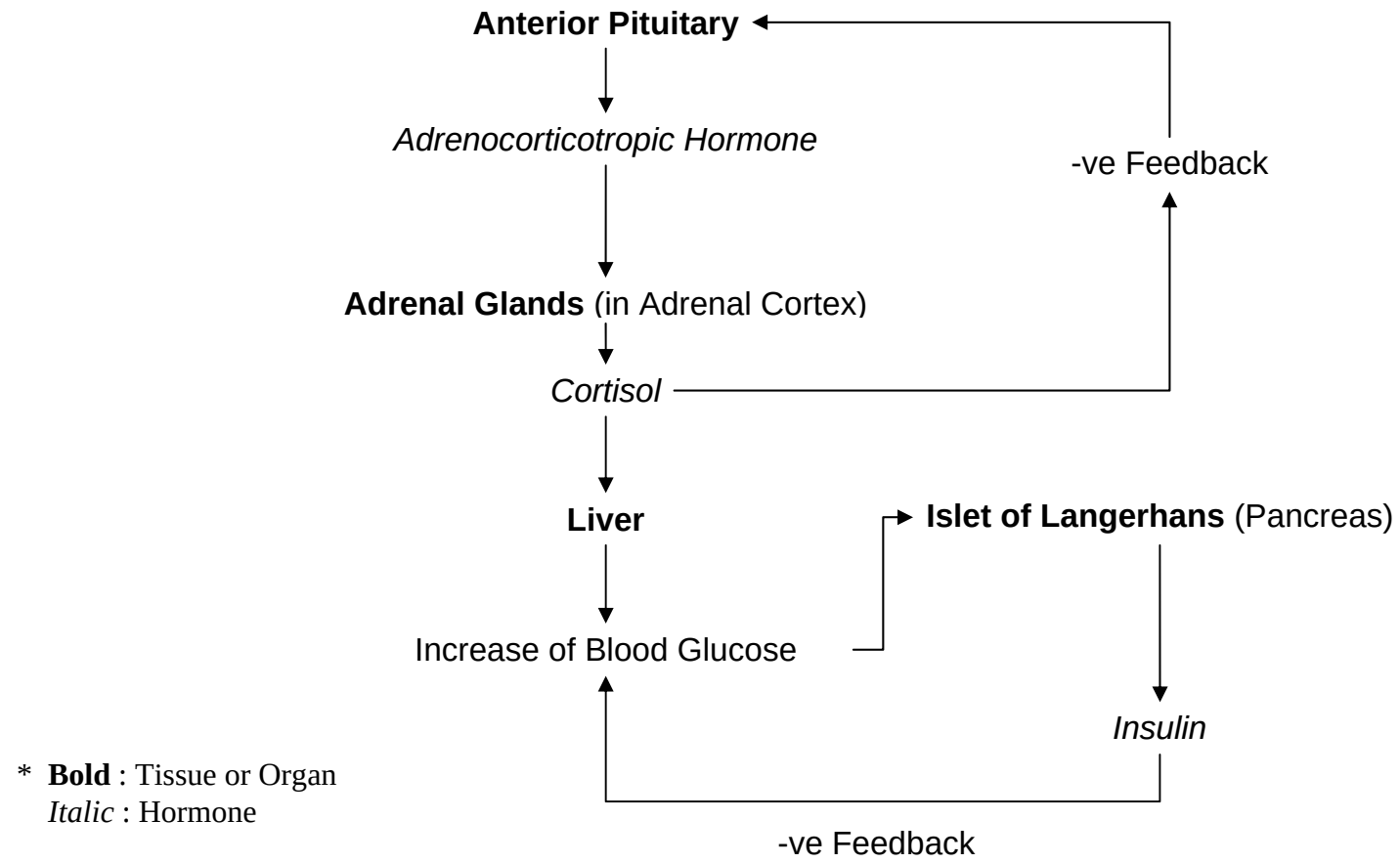

Figure 1-2. A diagram showing regulation of blood glucose concentration by pituitary gland and pancreas

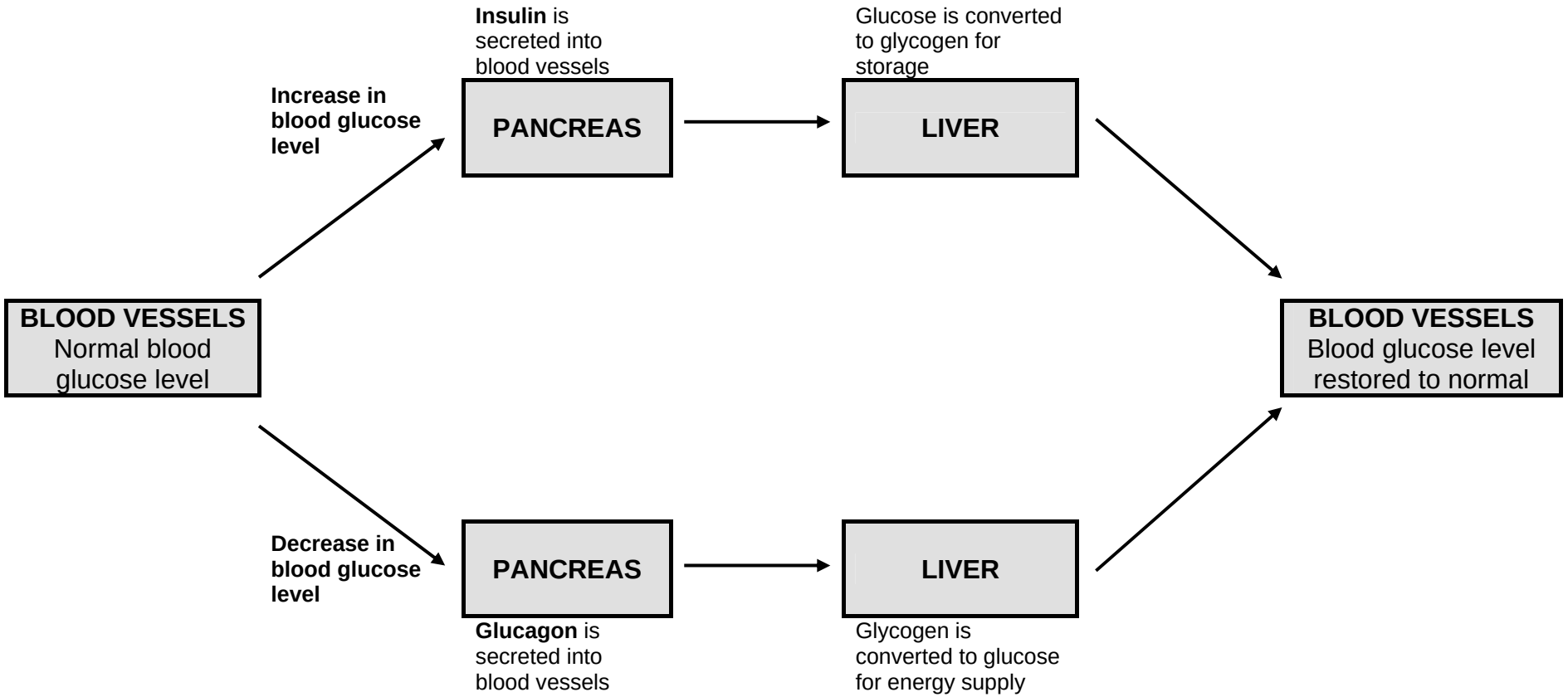

Figure 1-3. A simple diagram illustrating one of the hormonal regulation systems of blood glucose level

1.3 Research Objectives

The objectives of this research were to study the effects of essence of chicken on immunological response, the scavenging of free radicals and blood glucose in normal and burned rats. To achieve these aims, the biological parameters below were set.

1. Determine the serum IgG, IgA and IgM concentrations for immunological response.

2. Determine the plasma nitric oxide and lipid peroxidation levels for free radical scavenging capacity on EOC.

3. Determine serum glucose concentration.

1.4 Organization of Thesis

In Chapter 1, the purposes and research objectives of this study, and the background of EOC are described. The basic knowledge of the structure, origins and functions of immunoglobulins, free radicals and the basic functions of blood glucose and its regulatory system are also introduced. Chapter 2 will review the literature related to alterations of serum immunoglobulin concentrations, NO and LPO levels and blood glucose concentrations after burn injury. These reviews will serve as background information for the burn model used in this study. Chapter 3 will review past studies on the effects of EOC on immunological response, the scavenging of free radicals and blood glucose concentrations, which relate to the goals of this study. This study's design, details of the animal model and experiments and the adopted statistical

analysis will be described in Chapter 4. Details of the experimental work on the determination of serum immunoglobulin concentrations, plasma NO and MDA levels and blood glucose concentrations by various laboratory-based analytic methods will also be described in this chapter. In Chapter 5, the results of the analysis will be exhibited in tables and charts as mean values plus S.E.M. The results of the statistical analysis will also be given in this chapter. The results will then be discussed in Chapter 6, which will elaborate on the effects of EOC on immunological responses, free radical scavenging and blood glucose levels in normal and burned rats; the possible constituents responsible for the effects will be discussed as well as the clinical significances of the effects. Finally, the study will be concluded in Chapter 7. The implications of the results and the limitations of the study will be discussed, and further study directions will be suggested.

CHAPTER 2

LITERATURE REVIEW: CONSEQUENCES OF BURN INJURY ON BIOLOGICAL PARAMETERS

2.1 Introduction

Burn injury is a unique form of traumatic injury. Typical burn injury can be inflicted by scalds, flame, chemicals, electricity, radiation, contact and inhalation (Cook, 2002). Physiologically, it is clear that burn injury can greatly affect various biological functions and the processes of organs and tissues, such as impairing the integrity of skin, which allows the access of pathogens into the body, causes heat loss, disturbs body fluids and electrolyte balance, induces hypermetabolic rate, etc. In this chapter, the current literature on serum immunoglobulin concentrations, plasma NO and LPO levels and blood glucose level alterations after burn injury in human and animal models will be reviewed.

2.2 Alterations of Serum Immunoglobulin Levels after Burn Injury

It is widely accepted and evidenced that thermal injury can compromise various parts of the immune system. Undoubtedly, such immunosuppression leads to lowered resistance to infection and further increases the morbidity and mortality rate of burn victims. Hansbrough, Zapata-Sirvent and Peterson (1987) reported that burn injury can damage host non-specific and specific immune system, comprising humoral immunity and cell-mediated immunity.

Several studies have revealed that humoral immunity is prone to be compromised after burn injury in both human and animal models. Arturson *et al.* (1969) first noted that all immunoglobulin levels in serum and blister fluid dropped dramatically in patients with burn trauma. Immunoglobulin levels hit their lowest values 2-3 days postburn and rose to normal during weeks 1-2, while IgG took longer to restore to normal. In this study, the total body surface area (TBSA) of burn patients was not revealed and the sample size was relatively small (n=15), however, this study has already been used as a foundation for further studies on burn-related immunosuppression.

The findings of Arturson and colleagues provoked several responses in the years after they were published. Kohn and Cort made a prompt response in 1969. Apart from their essential results, which parallel Arturson *et al.*'s findings, they further observed that IgG levels fell much more profoundly than IgA and IgM in burn patients: because IgG is the smallest immunoglobulin, it leaks out first due to increased vascular permeability. In the same year, similar findings were reported by Ritzmann *et al.* (1969). Their results showed that the concentrations of serum IgG, IgA and IgM in burned children decreased rapidly and reached a nadir 2-5 days postburn. The only difference from Arturson *et al.*'s findings was that the immunoglobulin levels of the young patients returned to the control mean level later than those of the adult patients, especially the IgG level. This means that children experience more severe and prolonged IgG deficiency after burn injury. However, this conclusion was confined to juvenile burn patients and the TBSA of the burn wound was not described.

Studies on alterations of immunoglobulin levels after burn injury did not stop with these findings. In 1970, in further explorations, Munster, Hoagland and Pruitt reported that immunoglobulin levels were normal in the first few hours following burn injury and dropped rapidly to a maximum level of depression at 48 hours postburn. IgG levels were severely depressed during the postburn period and then rose to normal within 1-2 months. IgA and IgM levels were relatively unaffected, but the IgM levels changed sharply after fungal infection of the burn wound. Moreover, there was no statistically significant difference between the immunoglobulin patterns of burn wounds over or under 40% of TBSA. However, the age range of recruited subjects in this study was 5-62 years and the extent of burn varied from 5 – 90%. The large age and extent of burn differences between subjects may have affected the reliability of the results. These results were further confirmed by Leguit, Reerink-Brongers and Eijsvoogel (1973). Their study also reported that the patterns of serum IgG, IgA and IgM were the same in two patients with more than or less than 40% TBSA burn injury. In addition, their results showed that all immunoglobulin levels dropped initially and then rose to maximal levels at 4 weeks postburn for IgG and IgA, and 2 weeks postburn for IgM. The limitation of the study was that the intervals between each blood sampling were long (2 weeks). The entire picture of the immunoglobulin concentrations changes may not have been shown. Moreover, antibiotics were given to some patients, which may have influenced the results as well.

Bjornson, Altemeier and Bjornson (1977) studied differences in immunoglobulin level alterations between younger and older groups of burn patients. They found that the IgG and IgA levels were reduced significantly during the first 5 days postburn. However, the IgG and IgA levels of the older group were restored to normal within 6

to 10 days postburn and then remained normal for the ensuing period of the study; while in the younger group, the IgA level was restored to normal within 6 to 10 days postburn, but the IgG level was not fully restored to normal until 21-30 days postburn. The IgM levels of both the younger and the older group remained significantly low within the 40-day postburn period. However, as with the previous study there was a similar flaw: the difference in the extent of burn between subjects was large (2-70%) and errors in the results may have been amplified.

Similar results were noted by Ninnemann, Fisher and Wachtel in 1980. Within the study, both serum IgG and IgA levels initially showed depressions in the burn patients. Following the depressions, the IgG and IgA levels returned to supranormal concentrations. The authors predicted that such elevations were caused by antigenization to infection. Meanwhile, serum IgM levels increased postburn and the authors suggested that it may have been correlated with fungal infection. However, in this study, subjects with various types of burn injury, such as burn injury caused by flame, scald, electricity and inhalation were recruited, which may have influenced the immunoglobulin concentrations postburn. In 1982, Sengupta and colleagues also found that serum IgG, IgA and IgM levels tended to decrease after burn injury in 25 burned patients. Serum IgG levels fell to a nadir 3 days postburn, while IgA levels were depressed persistently below normal. The depressions of serum IgM levels were insignificant. The study further revealed that the depressions in immunoglobulin levels were related to the extent of burn, the period after burn and the type of immunoglobulin. The limitations of this study were the short duration of the experiment (12 days), which might not have been sufficient to entirely display the

changes in immunoglobulin levels; besides, the sample size was small (n=9) for the time sequence results after burn.

Hershman *et al.* (1988) pointed out that the responses of serum immunoglobulins to injury appear to depend on the type of injury, presence of infection, splenic function, and type of immunoglobulin. Their results demonstrated that there was a global reduction in serum levels of IgG, IgA and IgM in burn patients, but the results were not specific to burn patients, as patients with other traumatic injuries were also involved. Winkelstein (1984) reached a different conclusion concerning IgM levels postburn. According to his review, serum immunoglobulin levels decreased variably in burn patients - IgG and IgA were depressed during the first week postburn, while IgM was persistently depressed in some studies but elevated in others. In 1987, Hansbrough *et al.* also reported that IgG levels were greatly decreased in the first few weeks following burn injury, with a gradual return to normal levels over the ensuing weeks. On the other hand, the levels of IgA and IgM did not seem as severely affected.

In 1996, Bariar *et al.* observed that serum IgG, IgA and IgM concentrations dropped immediately following burn injury and reached their lowest values 3 days postburn. Both IgA and IgM levels dropped by half of their normal values. At 30 days postburn, all immunoglobulin levels returned to near normal. However, the age range of the subjects was too large (10-57 years), and thus may not have given representative results.

2.3 Alterations of Plasma Nitric Oxide Concentration and Lipid Peroxidation Level after Burn Injury

Horton (2003) and Halliwell *et al.* (1992) argued that tissue damage that results from burn injury can increase the oxidative stress on normal tissues in various ways. The mechanisms include elevation of radical-generating enzymes (e.g., xanthine oxidase) and/or their substrates, activation of phagocytes that can release additional free radicals (e.g., $O_2^{\cdot-}$), dilution and impairment of antioxidants and antioxidant mechanisms (e.g., SOD, catalase, glutathione, alpha tocopherol and ascorbic acid level), release of "free" metal ions that can facilitate free radical reactions, disruption of electron transport chains and increasing the leakage to form $O_2^{\cdot-}$, activation of phosphlipases, cyclooxygenases and lipoxtgenases and release of haem proteins. These support the idea that burn injury can exacerbate the formation of deleterious free radicals and their destructive activities to cells.

Manuel do Rosário Caneira da Silva and colleagues (1998) measured changes in plasma NO_2^- and NO_3^- levels in 27 burned patients. They noticed that there was a significant increase in plasma NO_2^- and NO_3^- levels on day 1 postburn and that there was no relationship between plasma NO levels and the TBSA of the burn wounds. However, some flaws were found in this study, including large differences in the age and extent of burn between subjects and that the subjects of the control and experimental groups were not matched by age. Another study in Japan investigated plasma NO_2^- and NO_3^- level changes in 19 burn patients. The results were perfectly comparable to the previous study - the investigators also found that plasma NO_2^- and NO_3^- rose immediately postburn when compared to non-burn volunteers, and that

there was no correlation between plasma NO_x levels and the TBSA of the wounds. In addition, they found that the plasma NO_x levels were significantly related to age (Saitoh *et al.*, 2001). However, the results of this study were compromised because some of the subjects suffered complications from inhalation injury and there were considerable differences between subjects in age and extent of burn. Lestaevel and colleagues (2003) also reported that NO concentrations in blood elevated significantly at 24 hours postburn in a burn rat model, compared with sham-burned rats. However, in the same study, the catabolic products of NO, blood NO_2^- and NO_3^- levels were significantly increased 6 hours after burn injury. All remained significantly high over 168 hours (7 days). The limitation of this study was that the experimental time was short (168 hours after burn injury), therefore, the time needed for NO levels to return to normal was not investigated. Similar findings were obtained by Hoşnuter *et al.*, (2004). They found that NO levels increased remarkably on day 1 postburn in rats, and then remained above normal up to 14 days postburn. In Onuoha, Alpar and Jones' (2001) study, 15 burn patients with 15% TBSA full thickness burn wound and 15 control subjects were employed. Plasma NO levels at 24 hours postburn were measured and the results showed that severe burn injury in patients was associated with a significant increase of plasma NO levels. However, the age, extent of burn and sex of the burn patients and control patients were not matched in this study.

For LPO, a study was done on a burned rat model by Centinkale and colleagues in 1997. In the study, plasma MDA levels were compared between the control group and the burn group. Each contained 12 rats and each animal in the burn group was inflicted with a 30% TBSA full thickness scald wound. The results showed that the burn injury induced a 57% rise of MDA in plasma at 24 hours after burn injury, which

indicated a significant increase in the LPO level. Similar findings were noticed by Centinkale *et al.* in 1999. The procedures were similar to the previous study, except there were 10 rats in the sham group and 16 rats in the burn group. The results also showed that the plasma MDA levels of the burned rats were elevated at 24 hours in comparison with the sham group, but the alterations were insignificant this time. The reliability of the results of both studies could be improved by increasing the sample size.

For changes in LPO level over the postburn period, Hoşnuter *et al.* (2004) performed an experiment using a rat model. Each rat in the experimental group was inflicted with a 20% TBSA full thickness scald wound. They found that the MDA levels increased significantly on day 1 postburn and remained above normal after the 14th day, but the age, extent of burn and sex of the burned patients and control animals were not matched in the study. Demling and LaLonde (1990a and 1990b) conducted two animal studies in which sheep were studied. Both studies concurred that plasma MDA concentrations increased remarkably in the burned sheep in comparison with those in the sham group. However, a sheep model is not usually used to represent humans.

For clinical studies, Nagane, Bhagwat and Subramanium (2003) showed the elevation of MDA level in patients with different degree of burn trauma. In this control-experimental study, 60 patients with burn injuries were recruited in the burn ward of a hospital, whilst the control group was formed by 25 healthy subjects with age and gender matched. Then the burned patients were divided into three groups according to their degree of burn injury. Whole blood samples were collected at the time of

admission and the MDA level was measured by colorimetric method. The results revealed that the lipid peroxidation level of the burned patients elevated significantly when compared to the healthy subjects ($p<0.001$). Moreover, the results showed a trend of elevation in lipid peroxidation level with the degree of severity of burn injury. The study confirms that burn trauma can elevate lipid peroxidation level in human being and exacerbate the postburn conditions through increasing the free radical activities and oxidative stress of the tissues.

Woolliscroft and colleagues (1990) investigated alterations of LPO levels in seven burn patients with more than 20% TBSA burn wounds. Investigators encountered increased conjugated diene (CD) concentrations, one of the lipid peroxides in blood, on day 1 postburn, and noted that the concentrations remained high up to day 5. However, the sample size was small (n=7). Another clinical study also showed that the plasma CD concentrations of burn patients (10-40% TBSA burn) were slightly higher than those of controls (Pintaudi *et al.*, 2000). Kumar and colleagues (1995) also revealed that serum LPO levels increased remarkably in 25 burn patients. Their results showed that MDA levels increased immediately postburn and then declined slightly, but still remained above normal through 10 days after burn injury. Age and sex were matched between burn and healthy control subjects, however, the experimental duration was not long enough to show whole changes of MDA postburn (10 days after burn injury).

2.4 Alterations of Blood Glucose Concentration after Burn Injury

Severe burn injury can cause metabolic and harmonic disturbances to the regulation of blood glucose concentration. The major characteristics of the metabolic changes of human burn patients include increased energy expenditure, increased protein catabolism and impairment of glucose metabolism (Herndon and Tompkins, 2004; Jahoor *et al.*, 1988; Matsuda *et al.*, 1987; Milner *et al.*, 1994; Wilmore *et al.*, 1974). According to the studies of Jahoor *et al.* and Matsuda *et al.*, the resting energy expenditure (REE) of burn patients elevated 27–50% when compared to their basal energy expenditure (BEE). Meanwhile, the investigators further noted that the rate of REE elevation depends on the TBSA of the burn wound.

Hypermetabolic response after burn injury is the consequence of the stress response in terms of hormone release and inflammatory mediator activation. Such metabolic abnormalities have to be rectified as they may impair immune responses and delay wound healing (Sim, 2002).

Burn injury can cause an imbalance in blood glucose regulation by impairing related hormonal systems. In 1974, Wilmore and colleagues found that the glucagon levels of 11 male burn patients increased immediately postburn, even when intravenous glucose had been given. At the same time, insulin levels tended to be lower than normal. However, patients were not proven to be free of diabetes prior to the experiment. Such hormonal complications can facilitate glycogen catabolism. Burn injury can play a role in promoting gluconeogenesis and increase the insulin

resistance of patients: all of these changes in the blood glucose regulation system are subsequent to the development of burn-related hyperglycaemia (Holm *et al.*, 2004).

Hyperglycaemia is a part of the hypermetabolic stress response. The term “diabetes of injury”, “stress diabetes” and “traumatic diabetes” are used to describe this state. Burn injury or critical illness turn on hyperglycaemia by activating the hypothalamic-pituitary-adrenal axis, which in turn increases hepatic glucose production and inhibits insulin-mediated glucose uptake to skeletal muscle. It has been suggested that hyperglycaemia during traumatic injury serves two purposes. The first is to replace volume loss either by enhancing the movement of intracellular fluid into intravascular space or by releasing water bonded to glucogen; the second purpose is to secure sufficient blood glucose supply to the brain, phagocytes and reparative cells (Mizock, 2001). However, Holm and colleagues (2004) suggest that there might be a certain degree of correlation between early hyperglycaemia and the subsequent mortality of burn patients. On the other hand, severe injury can enhance whole-body uptake of glucose in order to support non-insulin-mediated glucose uptake (NIMGU) and synthesis of immunocompetence (Mizock, 2001). Therefore, stable and adequate blood glucose levels have to be carefully maintained in burn patients.

2.5 Conclusion

After reviewing these studies, we can conclude that immunological responses after burn injury are remarkably depressed because of the global depression of serum IgG, IgA and IgM concentrations. Usually there is a more profound reduction in serum IgG level than in the IgA and IgM levels. Moreover, all immunoglobulin levels reach a nadir 3 – 5 days postburn and then start to return to their normal or supranormal

levels. Serum IgG level may take 2-4 weeks to be restored to its normal state, while IgA and IgM may take even longer. On the other hand, the antioxidative and reparative systems of the body are vulnerable to burn trauma; their collapse as well as the activation of some free radical-producing enzymes and pathways lead to increases in both nitric oxide and lipid peroxidation levels postburn. For blood glucose, burn injury impairs the hormonal blood glucose regulatory system of the body and results in a burn-related hypermetabolic state and hyperglycaemia. All of the above abnormalities that follow burn injury eventually jeopardize wound healing.

CHAPTER 3

LITERATURE REVIEW: EFFECTS OF ESSENCE OF CHICKEN ON THE IMMUNE SYSTEM, FREE RADICALS AND BLOOD GLUCOSE

3.1 Introduction

Several studies have described beneficial effects from EOC ingestion on various aspects of health (please refer to Chapter 1). The focus of this chapter is a review of the literature on the effects of EOC or its components (such as the two major dipeptides in EOC, carnosine and anserine) on immune function, free radical scavenging and blood glucose level. The roles and importance of dietary nutrients in the modulation of immune function after burn injury will be discussed as well.

3.2 Essence of Chicken, Chicken Soup and Immunological Responses

Prior studies have provided evidence of the benefits of EOC on health, whether in clinical or in animal models. However, there is still a lack of studies on the effects of EOC on the frontline defence system of the body, the immune system.

One study was carried out by Candlish in 1998. The investigator reported that EOC had a stimulatory effect on human circulating neutrophils. Neutrophils are the mainstay leukocytes in the immune system, which possess a phagocytic function and activate bactericidal mechanisms. They are the most numerous and important cellular

component of the innate immune response (Janeway *et al.*, 1999). In the study, the levels of superoxide and interleukin-1β were used as bio-markers for the activation of neutrophils. The investigator found that there were some high and some low molecular weight fractions of EOC that might act as activating factors for neutrophils, which may have stimulatory effects on the immune system. However, controversy persisted despite these results because EOC can also act as a superoxide scavenger in the same system, therefore, the actual level of superoxide might be damped, allowing for a certain degree of inaccuracy (Candlish, 1998). Moreover, this study was performed in vitro, thus it might not have been capable of reflecting the entire picture of immunological responses after the consumption of EOC, since the in vivo situation is expected to be more complicated.

In contrast, a study in 2000 by Rennard and colleagues investigated the effects of home-made chicken soup on immune functions. This study stated that chicken soup demonstrated an inhibitory effect on the chemotaxis of neutrophils (migration of neutrophils from blood vessels into tissues). This mild anti-inflammatory effect was beneficial in the mitigation of symptomatic upper respiratory tract infections. However, the inhibition of neutrophil chemotaxis can also be implemented as a kind of inhibition of the immune system as well. The study further pointed out that even chicken soup alone had an inhibitory effect on neutrophil chemotaxis, and that the effect varied greatly in commercial soups.

A folk wisdom tells us that it is helpful to take a cup of hot chicken soup when catching a common cold. It can help overcome the cold and unclog the nasal passages faster. Chicken soup usually contains chicken meat, fat and vegetables, such as

carrots, onions and corn. The solid components may be varied in different communities or countries. Although chicken soup is not exactly the same as EOC in terms of ingredients and manufacturing process (EOC is made of pure chicken with fat removed), their main constituent is chicken. Therefore, EOC may also have such a potentially inhibitory effect. However, further research is needed since Rennard *et al.*'s report is inconclusive for EOC. The results of the two studies demonstrate that as an indicator of immunological response, the activities of neutrophils demonstrate two extreme ends; therefore, conclusions on the effects of EOC on immunological response remain flawed and incomplete and a new indicator may be needed. The current in vivo study investigated the effects of EOC on immunological response by means of another bio-marker, concentrations of serum immunoglobulins.

3.3 Review on the Supply of Dietary Nutrients to Increase the Immune Function after Burn Injury

Several studies have noted that the levels of IgG, IgA and IgM in serum drop following profound tissue injuries, including injuries in both human and animal burn models (Arturson *et al.*, 1969; Bariar *et al.*, 1994; Bjornson *et al.*, 1976; Munster *et al.*, 1970; Ninnemann, 1982; Sengupta *et al.*, 1980; Winkeldtein, 1984). Such depressions in immunoglobulin levels impair the immune capabilities of patients and as a consequence compromise their ability to resist postburn infections, which further increases postburn morbidity and mortality (Warden and Ninnemann, 1981). Some studies have illustrated that changes in the hormonal environment after thermal injury create conditions that make subjects prone to increases in proteolysis and nitrogen loss (Rolandelli *et al.*, 1998; Saffle and Hildreth, 2002). Therefore, in the event of

injury, it has been suggested that adequate quantities of protein and amino acids must be supplied in order to satisfy ongoing metabolic demands and provide amino acids for protein synthesis. This would support the production of proteins, such as immunoglobulins that are expected to promote immunocompetence and hence recovery following severe burn injury (Peck and Chang, 1999; Saffle and Hildreth, 2002).

It is well documented that manipulation of the quality and quantity of dietary nutrients can alter several aspects of the immune functions in both normal and trauma patients (Chandra and Kumari, 1994; Biffl, Moore and Haenel, 2002; Kubena and McMurray, 1996). The effects of nutrition on the immune system include altering the rate of cell proliferation and protein synthesis, and the critical metabolic pathways influenced by individual nutrients and enzymes in immune responses that require specific micronutrients for optimal function (Chandra and Kumari, 1994). Therefore, it is anticipated that providing burn subjects with nourishing nutrient support would up-regulate impaired immune functions, which would reduce susceptibility to infection.

During the last two decades, the concept of using dietary nutrients to reduce the immunosuppression associated with trauma, surgery or critical illness has become popular (Ochoa and Ford, 2002). So-called immune-enhancing diets have no fixed formula, but are typically rich in proteins and amino acids (such as arginine, glutamine, omega-3 fatty acids and nucleotides). It is well documented that EOC is abundant in proteins and amino acids extracted from chicken meat (Matsumura *et al.*, 2002), which may provide a dietary source of protein after severe thermal injury. Candlish (1998) also demonstrated that EOC contains both a high and a low

molecular weight dipeptide (the low one is possibly carnosine), which can activate human circulating neutrophils. These studies support the proposed role for EOC in enhancing the synthesis and activation of immune system components.

3.4 Effects of Essence of Chicken on Free Radical Scavenging

Up to now, only one study has investigated the effects of EOC on free radical scavenging, and the effects on free radical scavenging of other products derived from chicken meats, such as chicken soup, are still unidentified.

The only study was carried out by Candlish in 1998. It was divided into two parts: the first part investigated the effects of the two dipeptides from EOC – carnosine and anserine, on their free radical scavenging effects on the artificial pyrogallol autooxidation system (PAS). In the second part of the experiment, the EOC was separated into high and low molecular weight fractions by dissolving it into 70% alcohol. This yielded an alcohol-soluble fraction and an alcohol precipitate fraction, which were the high and low molecular weight fractions respectively. The two fractions with neat EOC were then tested for their free radical scavenging capacity in the PAS as well. According to the results, both two major dipeptides from EOC showed free radical scavenging effects in the PAS, with anserine being more effective than carnosine. Moreover, the other part of the experiment revealed that both EOC fractions have free radical scavenging effects in the PAS as well, and an additive effect was noticed in neat EOC as it contains both fractions, which means that EOC has a more effective scavenging effect.

Although the study demonstrated that EOC has potential in free radical scavenging, there were some flaws in this study. First of all, the experiment was done in an artificial superoxide generating system, which may not reflect the more complicated in vivo environment. Besides, there may be some alterations to the free radical scavenging effects of EOC after it reacts with biological fluids and enzymes during digestion. Second, the PAS can only generate homogenous ROS, and the effects of EOC on various free radicals are still unknown. Therefore, this study was designed to supplement the previous work.

3.5 Effects of Essence of Chicken on Blood Glucose Concentrations

The effects of L-carnosine, one of the components of EOC, on the hyperglycaemia induced by intracranial injection of 2-deoxy-D-glucose (2DG-hyperglycaemia) were studied by Yamano and colleagues in 2001. They found that L-carnosine is effective in suppressing 2DG-hyperglycaemia in a rat model, and that it works in a dose-dependent manner. In the second part of the study, changes of plasma insulin and glucagon over time were determined after injection with L-carnosine. The results suggested that the suppressive effects of L-carnosine on 2DG-hyperglycaemia are due to increasing the plasma insulin level and decreasing the plasma glucagon level. Hence, it is possible that L-carnosine is an endogenous control factor of blood glucose level through autonomic nerves via the H3-receptor.

Nevertheless, L-carnosine is thought to be useful in down-regulating induced hyperglycaemia. Because EOC is a mixture of dipeptides from chicken meat, the interactive effects remain unclear.

3.6 Conclusion

Because there were flaws in the reviewed study on the effects of EOC on immune cell neutrophils, and a second study on chicken soup showed incompatible findings as well, the effects of EOC on immune response remain inconclusive. Moreover, past studies have confirmed that an increase in the supply of dietary nutrients is beneficial to the immune function of burn patients. Therefore, it is believed that EOC can provide extra nutrients to subjects with burn trauma and in turn enhance their suppressed immune function.

On the other hand, carnosine and anserine, the two main dipeptides in EOC, showed free radical scavenging actions in an artificial superoxide generating system. Carnosine also revealed modulating effects on induced high blood glucose concentrations in a rat model. However, this cannot fully represent the free radical scavenging capacity of neat EOC in vivo.

CHAPTER 4

MATERIALS AND METHODS

4.1 Introduction

In this chapter, the experimental design of this study, including the grouping of the rats and a rundown of the animal experiments, is illustrated. The detailed procedures of the animal model and the animal experiments, including holding, feeding, burn wound infliction and blood sampling, will also be given.

Following the animal experiments, the materials and detailed procedures of the immunological and biochemical analyses, which were carried out on the blood samples, are described. They include the determination of serum IgG, IgA and IgM concentrations using an enzyme-linked immunosorbent assay (ELISA), and plasma NO and LPO levels by Griess reaction and thiobarbituric acid reactive substances (TBARS) reaction respectively. The total nitrite concentration was analysed to represent the total NO level in plasma, whereas the LPO level was indicated by the concentration of its stable peroxide product, malondialdehyde (MDA). The blood glucose concentration was analysed by the automatic analyser, COBAS FARA II. Finally, the statistical analysis methods and software used in the study will be described.

4.2 Study Design

A three-group experimental design was used. An animal model of burn injury was employed for this study. One hundred and sixty-eight Sprague-Dawley rats were allocated randomly into three groups as shown in Figure 4-4. Their significance and sub-groupings are illustrated below:

1. UNBURN (Sham Group) – without challenge

The rats were not inflicted with a scald wound. They were further divided into three subgroups. One group was fed with 0.9% normal saline (NS) (n=24); one group was fed with 8.6% gelatin (GEL) (n=24); while the last group was fed with essence of chicken (EOC) (n=24). NS served as the placebo.

2. BURN (Experimental Group) – with challenge

The rats were inflicted with scald wounds. They were further divided into three subgroups and received normal saline (NS) (n=24), gelatin (GEL) (n=24) or essence of chicken (EOC) (n=24). NS was used as a placebo.

3. CONTROL

The rats received no treatment throughout the experiment (n=24).

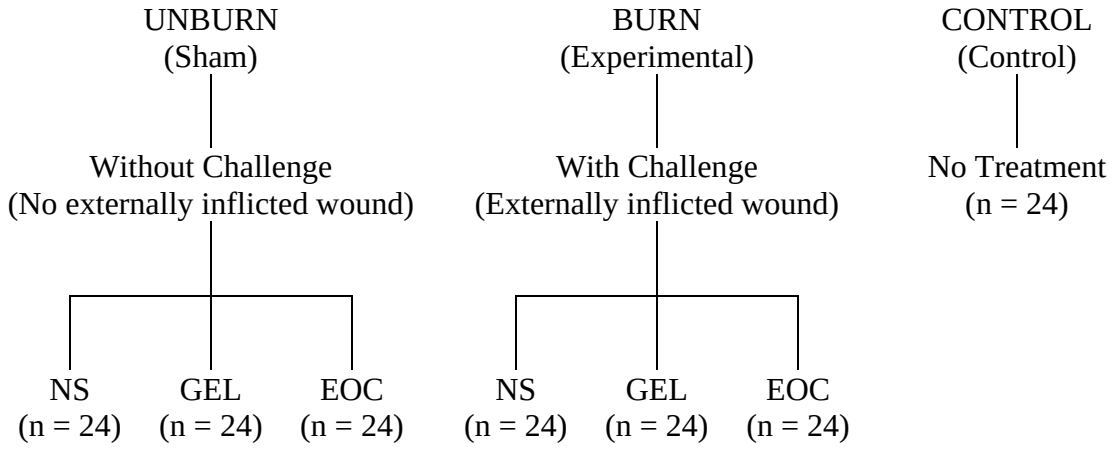

Figure 4-4. A diagrammatic illustration of the study design

All rats in the BURN group were scalded to induce an externally inflicted wound. Then a full-thickness scald burn with 30% Total Body Surface Area (TBSA) was inflicted on the rats at the beginning of the experiment (Day 0). All the rats in the UNBURN group received only a fake scald wound. The rats in the UNBURN group and the BURN group were fed with 0.9% normal saline (NS group), 8.6% gelatin (GEL group) or fresh undiluted EOC (EOC group) for 22 consecutive days according to their grouping. Blood samples were taken from all groups on days 1, 8, 15 and 22 for analysis.

4.3 Animal Experiments

One hundred and sixty-eight male Sprague-Dawley rats with an average body weight (BW) of 200 – 220g (six weeks old) were obtained from the Laboratory Animal Services Centre, The Chinese University of Hong Kong. They were housed in a separate room and caged individually. The room temperature was maintained between 18 – 22°C and the humidity was kept at 60 – 70%. The room was facilitated

with a 12:12 hours (0700-1900h) artificial light / dark cycle. The rats were allowed free access to standard commercial rodent chow and water. Prior to the experiment, all the rats were kept in a separate room for one week to adapt to the environment and for quarantine.

The right to conduct research with animal models and all procedures adopted in this study were licensed and approved by the Department of Health, The Hong Kong Government (License No.: (69) in DH/HKRO/G/5 Pt.27). Ethical practices were closely observed and monitored by the Animal Subjects Ethics Sub-committee, The Hong Kong Polytechnic University (Approval No.: 02/7).

4.3.1 Materials

The NS used is commercially available 0.9% normal saline (B. Braun Medical Industries, Penang, Malaysia). The GEL used was provided by Cerebos (Pacific) Limited in powder. The Gelatin (Byco M, Croda Colloids Ltd., Widnes Cheshire, England) is an enzymatically-hydrolysed fish collagen and rich in protein (almost 95% protein). It was dissolved in distilled water to an 8.6% in concentration to make its protein concentration similar to that of EOC. The bottled EOC (70ml/bottle, Cerebos (Pacific) Limited, Singapore) was manufactured in Thailand and provided by Cerebos. 0.76ml/kg BW of NS, GEL or EOC was fed to the rats according to their grouping. The EOC that was fed to the rats daily was undiluted and drawn from freshly opened bottles. A rat receiving 0.76ml/kg/day EOC for 22 days is approximately equivalent to a 70kg man consuming half a bottle (70ml) of EOC per

day (Xu and Sim, 1997). The solutions were administered directly into the oesophagus by use of a feeding needle (Harvard Apparatus, Massachusetts, USA).

4.3.2 Scald Infliction

Each rat in the BURN group was inflicted with a scald wound according to Walker and Mason's protocol (1968). First, the area to be scalded was calculated. Then the rat was anesthetized with sodium pentobarbital (50mg/kg BW, i.p.) (Abbott Laboratories, North Chicago, USA). The dorsum hair on the area to be scalded was shaved. The rat was fixed in the specially designed "burning device" (Figure 4-5, 4-6 and 4-7) and only the skin to be scalded was exposed through the aperture at the middle of the device. The exposed skin was immersed in 95°C water for 10 seconds to produce a III degree full thickness scald wound. On removal from water, the scalded area was dried by rolling on a sterilized towel quickly to avoid further burn. The rats were resuscitated with 0.9% normal saline (20ml/kg BW, s.c.). Finally they were released and caged. A hot lamp was used to reduce heat loss until the anesthetic wore off.

A sham scald wound was applied to the rats in the UNBURN group. The procedure for the sham scald infliction was similar to that inflicted on the BURN group rats, except that the exposed skin was immersed in 22°C instead of 95°C water. Buprenorphine (0.05mg/kg BW, s.c.) (Reckitt Benckiser Healthcare (UK) Limited, Hull, England) was given to the BURN group rats daily as an analgesic; the UNBURN group rats also received the analgesic to avoid possible differences caused by drug actions.

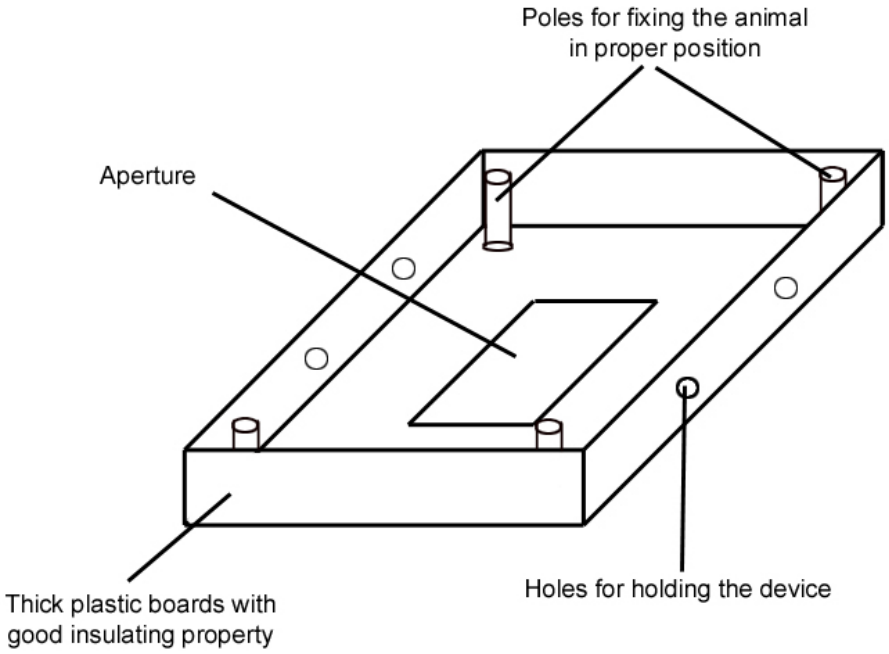

Figure 4-5. The design and structure of the modified burning device

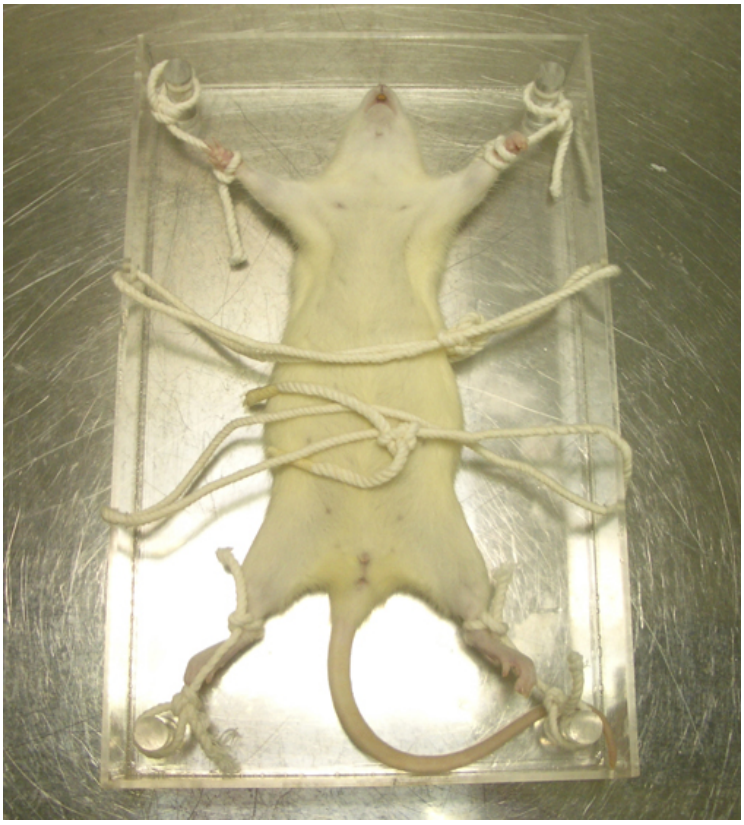

Figure 4-6. A photograph showing a rat was fixed on the burning device (Upper view)

Figure 4-7. A photograph showing a rat was fixed on the burning device (Bottom view)

4.3.3 Blood Sampling

Blood samples were obtained by cardiac puncture under anesthesia on days 1, 8, 15 and 22 of the experiment. The rats were then sacrificed after each blood taking. For serum collection, part of the collected blood samples was allowed to clot in plastic tubes at room temperature for one hour, while the remaining samples were put into heparin tubes (VACUETTE, Greiner bio-one, Kremsmünster, Austria) for the collection of plasma. Both the clotted blood and the heparinized blood were centrifuged at 3000rpm for 10 minutes. The serum and plasma samples were transferred to micro-tubes and stored at –30°C until analysis.

4.4 Determination of Serum IgG, IgA and IgM by Enzyme-linked Immunosorbent Assay

4.4.1 Materials

The coating buffer (50mM sodium carbonate solution, pH 9.6) contained 1.59g of sodium carbonate (Na_2CO_3) and 2.94g of sodium hydrogen carbonate ($NaHCO_3$) per litre. The washing buffer was phosphate buffered saline (PBS) (pH 7.4) with Tween 20. It contained, per litre, 1.33g of potassium phosphate dibasic (K_2HPO_4), 0.40g of potassium phosphate monobasic (KH_2PO_4), 8.77g of sodium chloride (NaCl, BDH Chemicals, UK) and 0.50ml of Tween 20. The blocking solution was tris-buffered saline (TBS) with 1% bovine serum albumin (BSA). It is commercially available in powdered form, and each pack was dissolved in 1 litre of distilled water before use. The peroxidase substrate buffer, pH 5.0, contained 19.21g of citric acid and 28.40g of sodium phosphate dibasic (Na_2HPO_4). The substrate solution was prepared by

dissolving 70mg of o-phenylenediamine dihydrochloride (OPD) and 20mg of urea-hydrogen peroxide in 100ml of peroxidase substrate buffer. All the chemicals were purchased from Sigma (Sigma-Aldrich Chemical Company, St. Louis, Missouri, USA) unless specifically mentioned.

4.4.2 Assay Procedures

The serum total IgG, IgA and IgM concentrations were measured by sandwich enzyme-linked immunosorbent assay (ELISA). The tests were based on the commercially available assay kits specifically designed for rat total IgG, IgA and IgM (Bethyl Laboratories Inc., Montgomery, USA). In brief, the anti-rat capture antibodies were diluted 1000 times with the coating buffer and each well of a 96-well plate (Greiner bio-one, Frickenhausen, Germany) was coated with 100µl of the antibodies overnight at 4°C. After incubation, the solution was removed and the plate was washed 5 times with the washing buffer. Then 200µl of blocking solution was added to each well and incubated at 37°C for 30 minutes before being removed and washed 5 times with the washing buffer. The wells were treated with 100µl rat serum (the serum was diluted with TBS with 1% BSA in 10^5 folds for IgG and IgM and 10^2 folds for IgA) and incubated at 37°C for 1 hour, then washed 10 times. 100µl anti-rat antibodies-horseradish peroxide (HRP) conjugated antibodies were added to each well, incubated at 37°C for another hour, and washed 5 times. The colour changing substrate solution was freshly prepared in 100ml of peroxidase substrate buffer (Chui *et al.*, 1989). 100µl of the substrate solution was added to each well and incubated for 30 minutes for colour development. Finally, 50µl of 4M sulphuric acid (H_2SO_4) was added to each well to stop the reaction and the plate was measured by microtiter plate reader at 450nm (SPECTRAFluor Plus, Tecan Group Ltd., Maennedorf, Switzerland).

Each sample was run in triplicate for all tests. The within-assay precision for the IgG test ranged from 3.49 to 8.49%CV (Percent Coefficient of Variation) and the lower limit of sensitivity was 7.8ng/ml. For the IgA test, the within-assay precision was 3.69 to 6.17%CV and the lower limit of sensitivity was 15.6ng/ml. For the IgM test, the within-assay precision was 4.46 to 15.15%CV and the lower limit of sensitivity was 31.25ng/ml. The purified anti-rat antibodies from the kits have no cross-reactivity with other immunoglobulins.

The calibration curves were constructed by serial dilution of the rat reference sera, which was provided in the kits. The concentrations of immunoglobulins in serum were expressed as mg/ml for IgG and IgM and μg/ml for IgA.

4.5 Determination of Plasma Nitric Oxide Level by Griess Reaction

4.5.1 Materials

The 50mM potassium phosphate buffer (pH 7.5) was prepared by dissolving 4.35g of K_2HPO_4 and 3.40g of KH_2PO_4 to 500ml distilled water separately. Then 30ml of KH_2PO_4 were added to 100ml of K_2HPO_4 solution, and 2ml volume units were slowly added until the pH was close to 7.5. For 0.11mM flavin adenine dinucleotide (FAD), 0.0456g of FAD was dissolved in 500ml distilled water. The 0.86mM reduced β-nicotinamide adenine dinucleotide phosphate (NADPH) was prepared by dissolving 0.358g of NADPH in 500ml of distilled water. Nitrate reductase (NAD[P]H) from

Aspergillus niger was brought to 1.0Unit/ml with distilled water. The Greiss reagent was the modified powder and commercially available; it was dissolved in fresh distilled water before use. All chemicals were purchased from Sigma (Sigma-Aldrich Chemical Company, St. Louis, Missouri, USA)

4.5.2 Assay Procedures

The determination of NO involved two steps. The first was the conversion of nitrate (NO_3) to nitrite (NO_2) by the action of nitrate reductase from *Aspergillus niger*. The second was that the nitrite in the samples reacted with the Griess reagent to form a coloured product, which could be quantified by the spectrophotometric method. For preparation, the plasma samples were filtered with a spin concentrator with a filter at 10,000 MWCO (Vivaspin 500, Vivascience, Hannover, Germany). In each well of the 96-well plate (Greiner bio-one, Frickenausen, Germany), 30µl of filtered plasma sample were then mixed with 20µl of distilled water, 20µl of 50mM potassium phosphate buffer, 10µl of 0.86mM NADPH, 10µl of 0.11mM FAD and 10µl of 1.0Unit/ml nitrate reductase. The plate was incubated for 1 hour at room temperature in the dark. Afterward, 200µl of Griess reagent was added to each well. The plate was incubated for an additional 10 minutes at room temperature. Finally, the absorbance was measured at 540nm by using a microtiter plate reader (SPECTRAFluor Plus, Tecan Group Ltd., Maennedorf, Switzerland).

Each sample was analyzed in triplicate. The within-assay precision of this test ranged 1.08 to 6.99%CV. The lower limit of sensitivity of this test was 2.5µM.

The calibration curve was constructed by dissolving the known concentrations of sodium nitrite (Sigma) in distilled water. The nitrite levels in the samples were calculated by subtracting the value of the enzyme blank and were expressed as μmol/l NOx in plasma.

4.6 Determination of Plasma Lipid Peroxidation Level by Thiobarbituric Acid Reactive Substances Reaction with Fluorometric Method

4.6.1 Materials

The 42mmol/l H_2SO_4, per litre, was prepared by adding 4.20ml of 98% H_2SO_4 into 995.80ml distilled water. The 10% phosphotungstic acid (w:v) contained 10g of phosphotungstic acid in 100ml distilled water. For 0.67% 2-thiobarbituric acid (TBA) (w:v), 6.7g of TBA were dissolved into 1 litre distilled water. It was then mixed with acetic acid in 1:1 (v:v) just before use. The 0.7mmol/l butylated hydroxytoluene (BHT) was prepared by dissolving 0.154g of BHT in 1 litre of distilled water and then mixing it with ethanol to a ratio of 1:4 (v:v). All chemicals were purchased from Sigma (Sigma-Aldrich Chemical Company, St. Louis, Missouri, USA)

4.6.2 Assay Procedures

Malondialdehyde (MDA) is a relatively stable intermediate of lipid peroxidation. It has been widely accepted as a marker to show the lipid peroxidation level. MDA can form a complex with thiobarbituric acid (TBA), which can be detected by a fluorescence detector.

In brief, 10µl of rat plasma sample was mixed with 500µl of 42mmol/l H_2SO_4 in a micro-centrifuge tube. Then 125µl of 10% phosphotungstic acid was added and vortex-mixed. The solution was allowed to stand at room temperature for 5 minutes and was then centrifuged at 4000rpm for 10 minutes. The supernatant was discarded and sediment was re-suspended in 300µl of 42mmol/l H_2SO_4 and 45µl of 10% phosphotungstic acid. The solution was centrifuged at 4000rpm for another 10 minutes. The supernatant was discarded again and the sediment was re-suspended in 350µl of distilled water. Then 50µl of 0.7mmol/l BHT and 100µl of 0.67% TBA reagent were added. The reaction mixture was heated at 95^{o}C for 1 hour in a water bath. After cooling with ice, the MDA$(TBA)_2$ complex was extracted with 500µl of n-butanol (Sigma). The mixture was shaken vigorously and centrifuged at 4000rpm for 15 minutes. Then 200µl of butanol layer was transferred to a black 96-well plate (Greiner bio-one, Frickenhausen, Germany) and taken for fluorometric measurement with a microtiter plate reader (SPECTRAFluor Plus, Tecan Group Ltd., Maennedorf, Switzerland), which was made at 515nm excitation and 553nm emission.

All samples were measured in triplicate. The within-assay precision of this assay was 2.31 to 14.62%CV. The lower limit of sensitivity of this test was 0.15µmol/l.

The calibration curve of MDA was established from 1,1,3,3-tetraethoxypropane (TEP) (Sigma). Plasma samples were replaced by known concentrations of TEP in the test and the amount of MDA was expressed as µmol/l.

4.7 Determination of Blood Glucose Concentration by the Automatic Analyzer – COBAS FARA II

4.7.1 Materials

The cuvette rotors were purchased from Roche Diagnostic System Inc., USA. Each rotor was used only once in the experiment, and it was replaced every time a new test was started. The calibrator serum, assayed control serum level 1 and 2 and glucose assay reagent were purchased from Biosystems, Spain.

4.7.2 Assay Procedures

All the rat serum samples, control sera and test reagent were loaded into the COBAS FARA II analyser (Roche Diagnostic System Inc., USA). After the test programme was set into the analyser, the tests were run automatically. The test is based on enzymatic and spectrophotometric techniques. The blood glucose was converted to a coloured complex through the two reactions below in the presence of two enzymes.

$$\text{Glucose} + \tfrac{1}{2}O_2 + 2H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconate} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

The colour change was then measured by the built-in spectrophotometer at 500nm. The analyser generated the report automatically and the glucose concentration was

expressed as mmol/l. The calibration curve was established and verified by the calibrator and control sera. All samples were measured in triplicate. The detection limit of this assay was 0.0126mmol/l and the sensitivity was 0.22mA·dl/mmol. The within-assay precision ranged from 0.9 to 1.2%CV.

4.8 Data Treatment and Statistical Analysis

Data was treated with the Statistical Package for the Social Sciences (SPSS) version 11.0 and the charts were plotted by Microsoft Excel 2002. Data is presented as mean with standard error of mean (S.E.M.). ANOVA and LSD posthoc test were used to indicate a significant difference between the NS, GEL and EOC groups of immunoglobulin levels, nitric oxide concentration, lipid peroxidation level and blood glucose concentration. The level of significance was set at $p < 0.05$.

4.9 Conclusion

To achieve the research objectives, the experimental study design and the burn wound inflicted animal model constituted the basis of this study. As discussed in Chapter 1, burn injury impairs the immunological response, increases pathogenic free radical production and causes an imbalance in blood glucose regulation in the rat model. The burned animal provided a good model for determining changes in the above biological parameters after ingestion of NS, GEL and EOC. In the meantime, a sham model with a fake burn wound and a control group with no treatment acted as a normal and a reference group respectively. The immunological and biochemical parameters were analysed by ELISA, Griess Reaction, TBARS reaction and COBAS FARA II. Finally, differences in immunoglobulin concentrations, free radical levels and blood glucose

concentrations between the NS-, GEL- and EOC-fed groups were analysed by ANOVA.

CHAPTER 5

RESULTS

5.1 Introduction

This chapter reports the findings with regard to changes in serum immunoglobulin concentrations, nitric oxide and lipid peroxidation levels, and blood glucose concentration after consumption of EOC. The results concerning the effects of consumption of the protein-rich GEL will also be reported. Within the chapter, the data are presented in mean values plus S.E.M., and ANOVA was used to find out the significant differences between the NS, GEL and EOC groups. The figures are presented in tables and line charts, and results from the UNBURN and BURN groups are presented in separate charts. The statistical analysis was done by SPSS version 11.0 and the charts were plotted by Microsoft Excel 2002. The significance level was set at $p<0.05$.

5.2 Effects of Essence of Chicken on Serum IgG, IgA and IgM Concentrations in Normal and Burned Rats

The statistical analysis showed that the serum IgG, IgA and IgM concentrations rose in both the UNBURN and BURN rat groups following the consecutive consumption of GEL and EOC on some postburn days (Table 5-4). In the UNBURN group, a significant elevation of serum IgG level was noticed on day 1 postburn in the GEL group and on day 8 in the EOC group ($p=0.026$ and $p=0.013$ vs. UNBURN-NS group respectively) (Figure 5-8). On the other hand, both the GEL and EOC groups in the

BURN group showed similar results on day 8 postburn ($p=0.001$ and $p=0.017$ vs. BURN-NS group respectively) (Figure 5-9).

Significant differences in serum IgA were found in both the GEL and EOC groups of the UNBURN group on day 15 (both $p<0.050$ vs. UNBURN-NS). GEL showed no statistical significance on up-regulation of serum IgA level in the UNBURN group (Figure 5-10). Surprisingly, significantly low levels of serum IgA were recorded in the BURN group on days 1 and 15 after the consumption of GEL ($p=0.038$ and $p=0.035$ vs. BURN-NS respectively). However, the opposite result was noticed on day 22: the serum IgA level of the BURN-GEL was higher than the corresponding BURN-NS group ($p=0.034$). For the BURN-EOC group, a significant high was found on day 22 ($p=0.029$ vs. BURN-NS) (Figure 5-11).

The serum IgM level of the UNBURN-GEL was significantly higher than the corresponding NS group on day 22 ($p=0.025$); for the UNBURN-EOC group, significant highs were recorded on days 1 and day 22 ($p=0.010$ and $p<0.050$ vs. UNBURN-NS respectively) (Figure 5-12), while in the BURN group, the serum IgM level of the EOC group was significantly higher than that of the NS group on day 15 ($p=0.040$). The BURN-GEL showed no difference in comparison to the BURN-NS group (Figure 5-13).

Table 5-4. The effects of NS, GEL and EOC on serum IgG, IgA and IgM concentrations in the UNBURN and BURN groups on days 1, 8, 15 and 22. CONTROL shows the normal level for each immunoglobulin in serum.

Type of Immunoglobulin	CONTROL (n=6)	UNBURN			BURN		
		NS (n=6)	GEL (n=6)	EOC (n=6)	NS (n=6)	GEL (n=6)	EOC (n=6)
IgG (mg/ml)							
Day 1	0.96±0.05	0.92±0.02	1.03±0.03 **	0.92±0.04	0.75±0.01	0.70±0.04	0.68±0.03
Day 8	0.95±0.04	0.96±0.05	1.07±0.01	1.12±0.04 *	0.67±0.03	0.90±0.05 *	0.83±0.05 *
Day 15	0.93±0.05	1.06±0.06	1.20±0.02	1.10±0.05	1.17±0.07	1.10±0.08	1.12±0.07
Day 22	0.93±0.06	1.03±0.03	1.05±0.04	1.10±0.04	1.07±0.04	1.13±0.04	1.12±0.03
IgA (μg/ml)							
Day 1	78.04±2.49	78.59±2.13	80.72±3.61	84.71±3.24	68.37±2.38	59.12±1.24 #	60.15±3.91
Day 8	78.80±4.59	78.63±3.87	76.74±2.96	77.53±3.32	64.38±3.23	68.91±2.73	69.92±3.42
Day 15	78.59±1.37	77.60±1.99	94.42±3.33 *	97.78±2.44 *	73.06±1.25	66.00±2.80 #	69.18±1.38
Day 22	78.57±5.21	76.15±0.96	80.49±2.67	71.88±1.87	66.30±1.17	77.65±3.78 *	78.02±2.88 *
IgM (mg/ml)							
Day 1	0.85±0.06	0.77±0.03	0.88±0.09	0.99±0.02 *	0.71±0.02	0.68±0.01	0.65±0.07
Day 8	0.73±0.05	0.85±0.05	0.93±0.07	0.94±0.05	0.69±0.02	0.80±0.03	0.80±0.04
Day 15	0.84±0.07	0.93±0.03	1.08±0.04	1.00±0.01	0.89±0.03	0.84±0.04	1.09±0.10 **
Day 22	0.85±0.07	0.95±0.07	1.21±0.05 *	1.39±0.13 *	0.87±0.04	1.01±0.07	1.08±0.05

· The serum immunoglobulin concentrations are expressed as mean ± S.E.M. (**) indicates that the immunoglobulin concentrations in this group were significantly higher than in the corresponding two groups ($p<0.05$); (*) indicates that the immunoglobulin concentrations in this group were significantly higher than in the corresponding NS group only, and ($^{\#}$) means the opposite ($p<0.05$). 6 rats were sacrificed at each specified time interval.

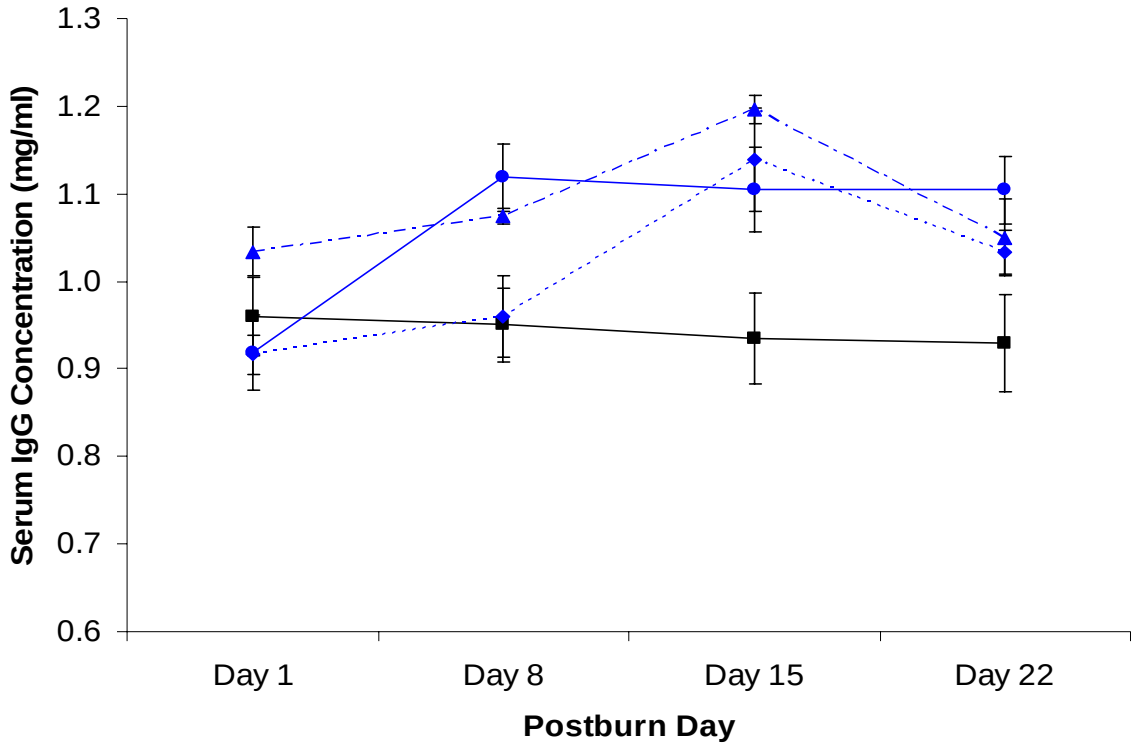

Figure 5-8. The serum IgG concentrations of the **UNBURN group** rats after intake of **NS** (◆, dot), **GEL** (▲, semi-solid) and **EOC** (●, solid). The **CONTROL group** (■, solid) is also shown as reference for the normal serum IgG concentration of rats.

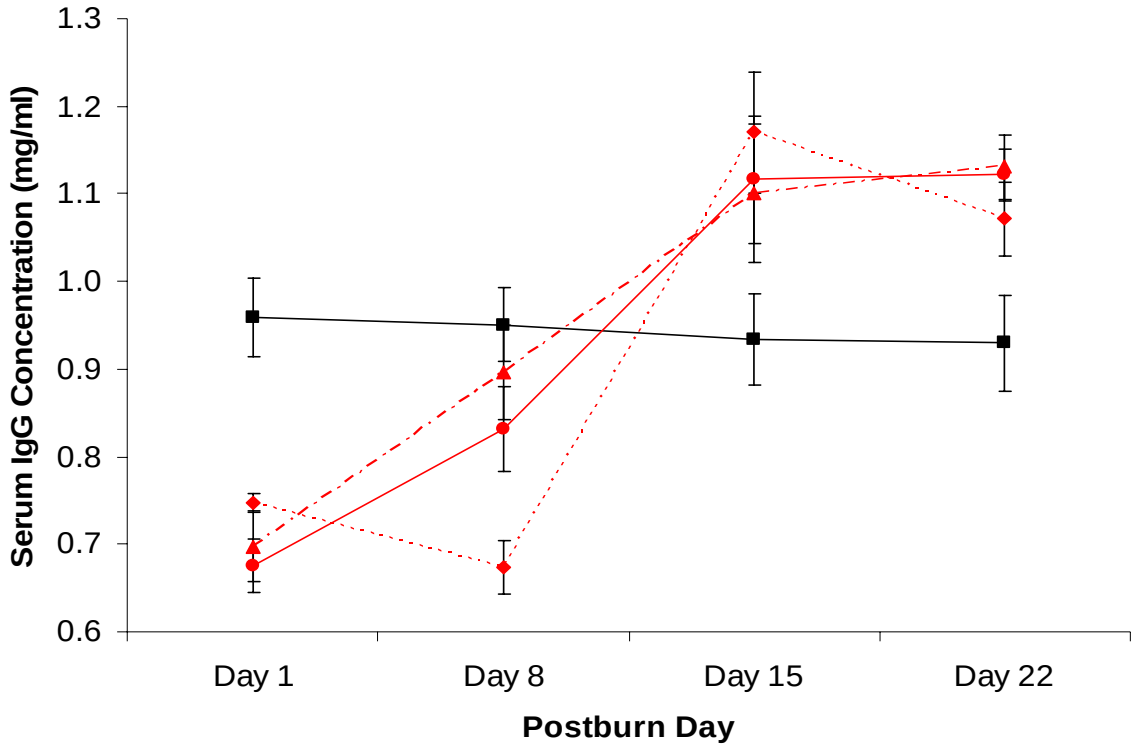

Figure 5-9. The serum IgG concentrations of the **BURN group** rats after intake of **NS** (◆, dot), **GEL** (▲, semi-solid) and **EOC** (●, solid). The **CONTROL group** (■, solid) is also shown as reference for the normal serum IgG concentration of rats.

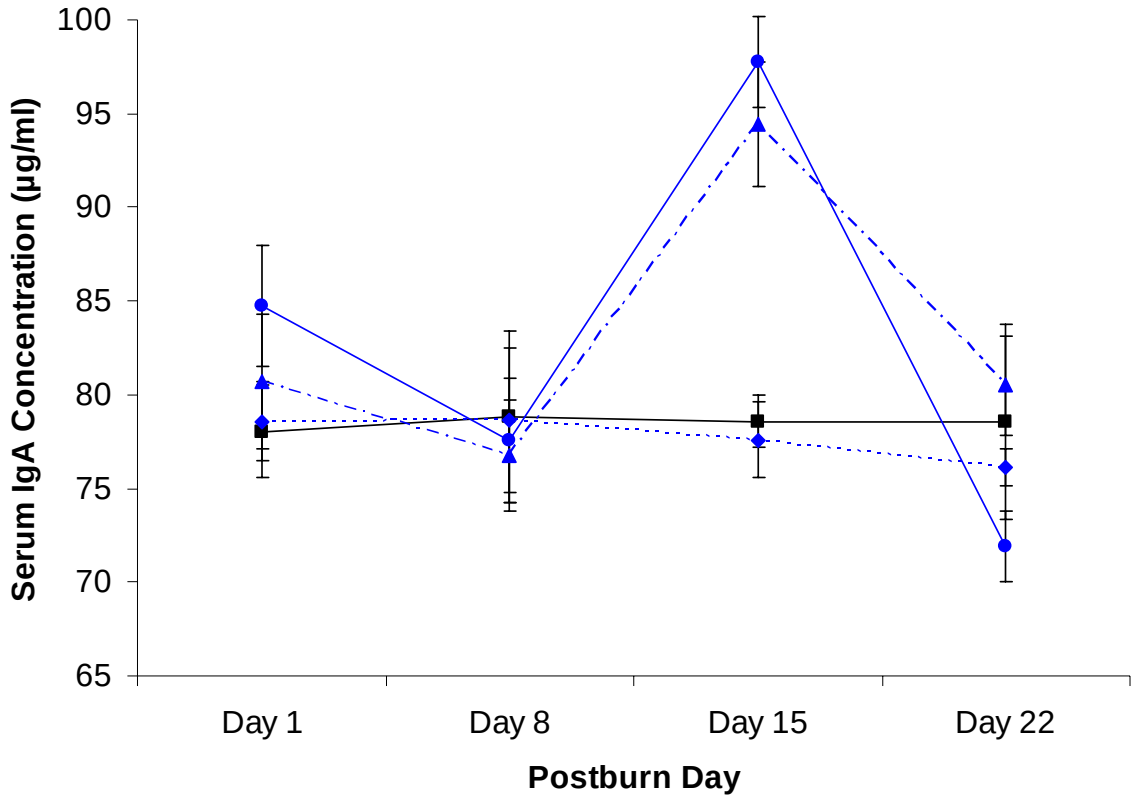

Figure 5-10. The serum IgA concentrations of the **UNBURN group** rats after intake of **NS** (◆, dot), **GEL** (▲, semi-solid) and **EOC** (●, solid). The **CONTROL group** (■, solid) is also shown as reference for the normal serum IgA concentration of rats.

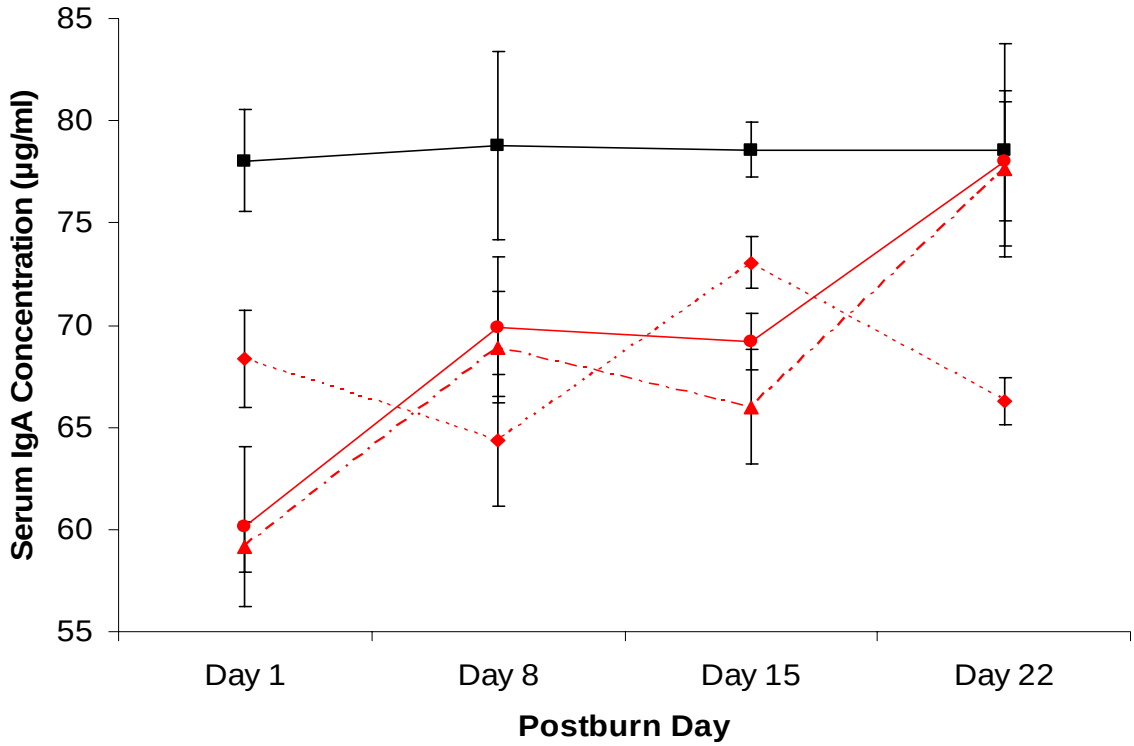

Figure 5-11. The serum IgA concentrations of the **BURN group** rats after intake of **NS** (◆, dot), **GEL** (▲, semi-solid) and **EOC** (●, solid). The **CONTROL group** (■, solid) is also shown as reference for the normal serum IgA concentration of rats.

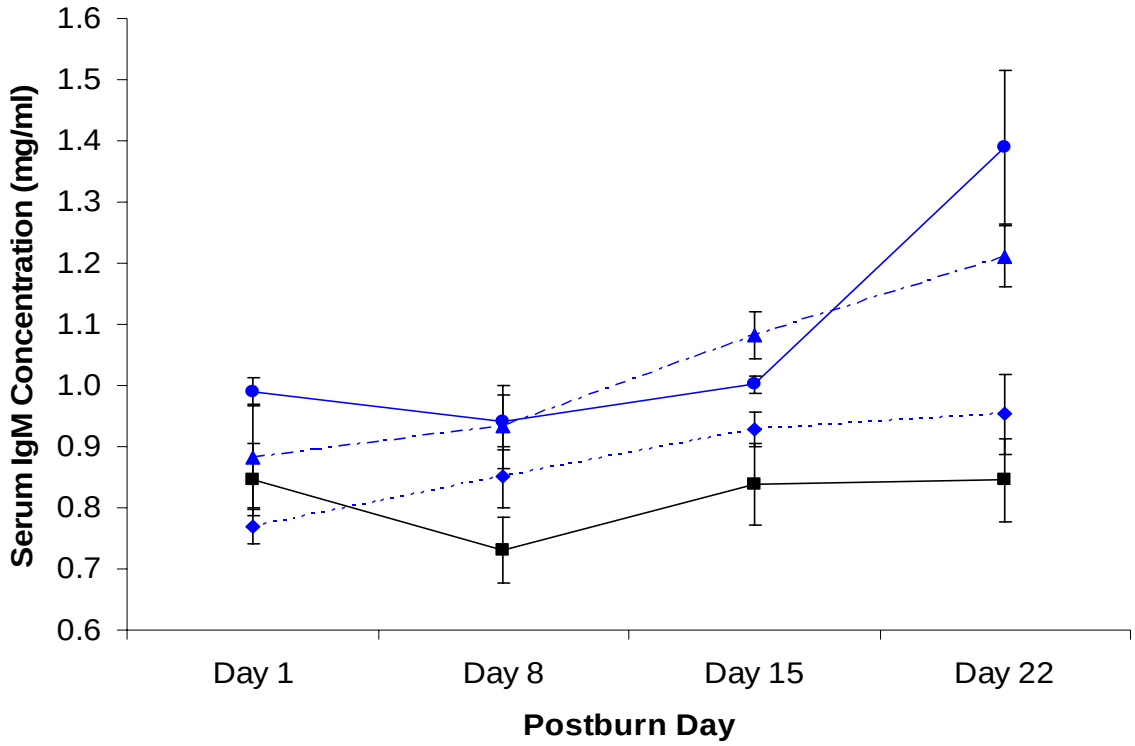

Figure 5-12. The serum IgM concentrations of the **UNBURN group** rats after intake of **NS** (◆, dot), **GEL** (▲, semi-solid) and **EOC** (●, solid). The **CONTROL group** (■, solid) is also shown as reference for the normal serum IgM concentration of rats.

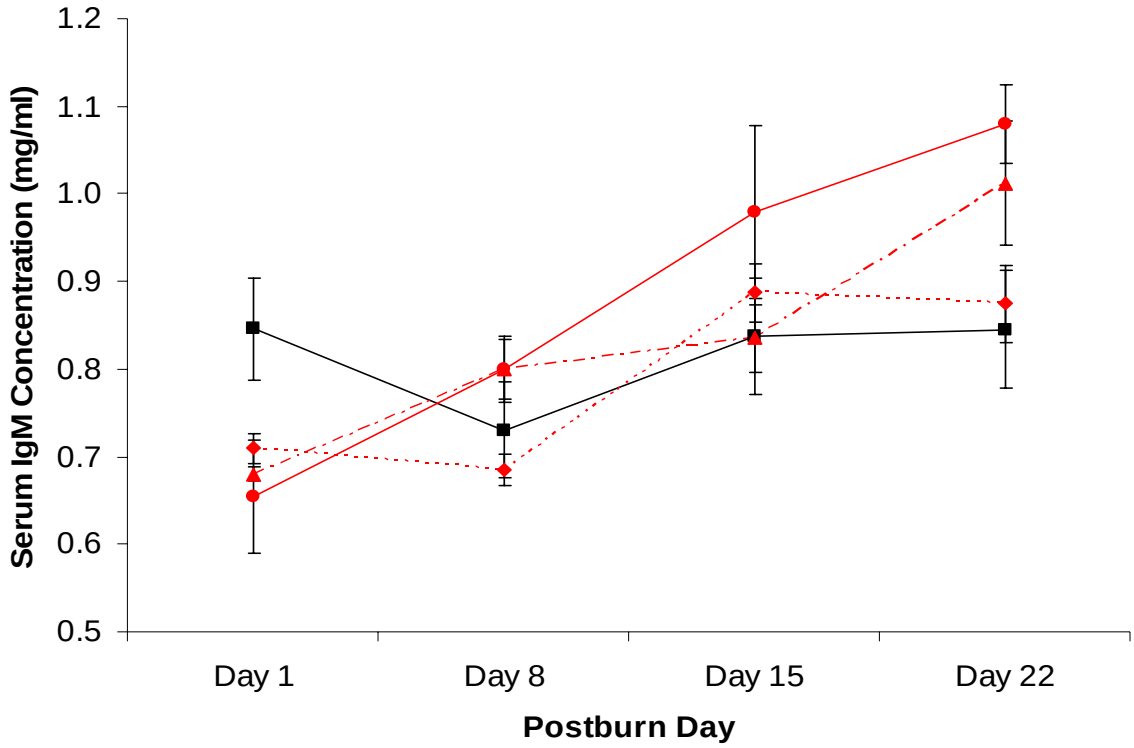

Figure 5-13. The serum IgM concentrations of the **BURN group** rats after intake of **NS** (◆, dot), **GEL** (▲, semi-solid) and **EOC** (●, solid). The **CONTROL group** (■, solid) is also shown as reference for the normal serum IgM concentration of rats.

5.3 Effects of Essence of Chicken on Plasma Nitric Oxide Concentration and Lipid Peroxidation Level in Normal and Burned Rats

After the consumption of GEL and EOC, there was no significant change in plasma NO concentration for the UNBURN group (Figure 5-14). By contrast, a significantly low plasma NO level was found on day 15 postburn for the BURN group, after the consumption of GEL and EOC ($p=0.024$ and $p=0.002$ vs. BURN-NS respectively) (Figure 5-15).

A significant fall in the plasma MDA level of the UNBURN-EOC group was noticed on day 8 in comparison to the corresponding NS group ($p=0.018$), whilst GEL did not have any effect on the UNBURN group (Figure 5-16). For the BURN group, a significant low plasma MDA level was observed on day 8 postburn after the consumption of GEL and EOC ($p=0.023$ and $p=0.006$ vs. BURN-NS respectively) (Figure 5-17).

Table 5-5. The effects of NS, GEL and EOC on plasma NO and MDA concentrations in the UNBURN and BURN groups on days 1, 8, 15 and 22. The CONTROL shows the normal level for NO and MDA in plasma.

	CONTROL (n=6)	UNBURN			BURN		
		NS (n=6)	GEL (n=6)	EOC (n=6)	NS (n=6)	GEL (n=6)	EOC (n=6)
Nitric Oxide (µmol/l)							
Day 1	18.31±0.99	26.89±1.88	26.45±5.16	22.34±2.37	48.75±3.87	41.12±3.64	40.00±4.96
Day 8	23.89±3.11	29.79±5.00	22.62±6.19	30.95±5.63	47.36±4.93	46.64±4.67	34.19±4.84
Day 15	21.69±3.15	24.64±4.00	27.73±3.84	27.49±4.48	46.56±2.21	33.44±4.59 ¯*	25.30±4.98 ¯*
Day 22	20.30±2.19	24.86±5.09	21.98±5.11	25.13±5.09	38.03±4.79	30.59±3.59	27.73±3.27
MDA (µmol/l)							
Day 1	2.37±0.16	2.33±0.27	2.44±0.20	2.30±0.18	2.77±0.13	2.46±0.41	2.66±0.19
Day 8	2.51±0.24	2.64±0.12	2.65±0.06	2.07±0.17 ¯**	3.36±0.13	2.79±0.19 ¯*	2.63±0.16 ¯*
Day 15	2.63±0.24	2.68±0.32	2.48±0.21	2.36±0.21	3.23±0.26	3.14±0.19	2.79±0.17
Day 22	2.59±0.13	2.53±0.26	2.59±0.16	2.47±0.15	2.79±0.22	2.72±0.29	2.84±0.17

· The NO and MDA concentrations are expressed as mean ± S.E.M. (¯**) indicates that the NO or MDA concentrations in this group were significantly lower than in the corresponding two groups ($p<0.05$); (¯*) indicates that the NO or MDA concentrations in this group were significantly lower than in the corresponding NS group only ($p<0.05$). 6 rats were sacrificed at each specified time interval.

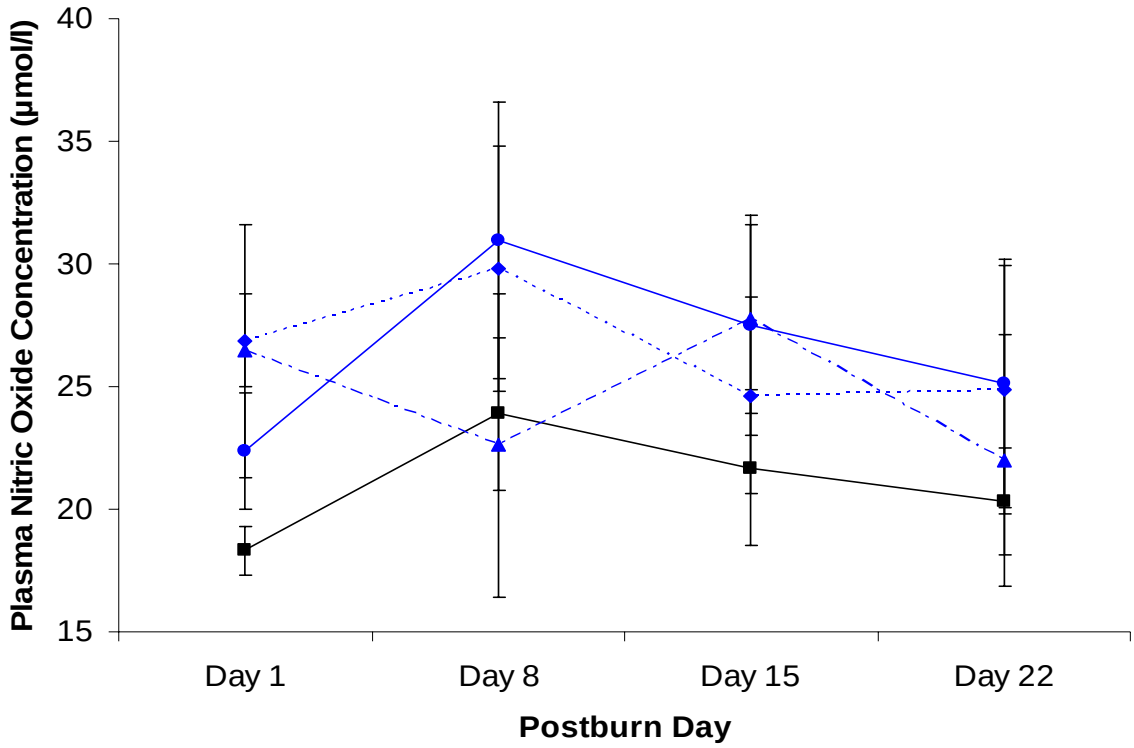

Figure 5-14. The plasma nitric oxide concentrations of the **UNBURN group** rats after intake of **NS** (◆, dot), **GEL** (▲, semi-solid) and **EOC** (●, solid). The **CONTROL group** (■, solid) is also shown as reference for the normal nitric oxide concentration of rats.

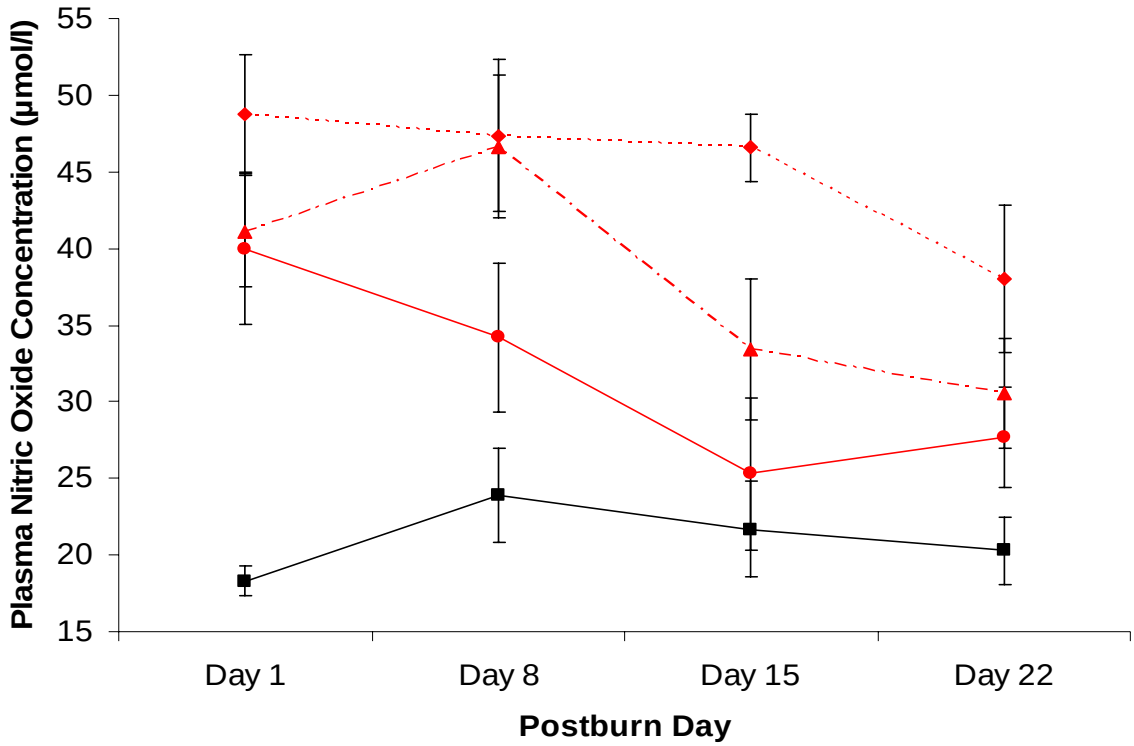

Figure 5-15. The plasma nitric oxide concentrations of the **BURN group** rats after intake of **NS** (◆, dot), **GEL** (▲, semi-solid) and **EOC** (●, solid). The **CONTROL group** (■, solid) is also shown as reference for the normal plasma nitric oxide concentration of rats.

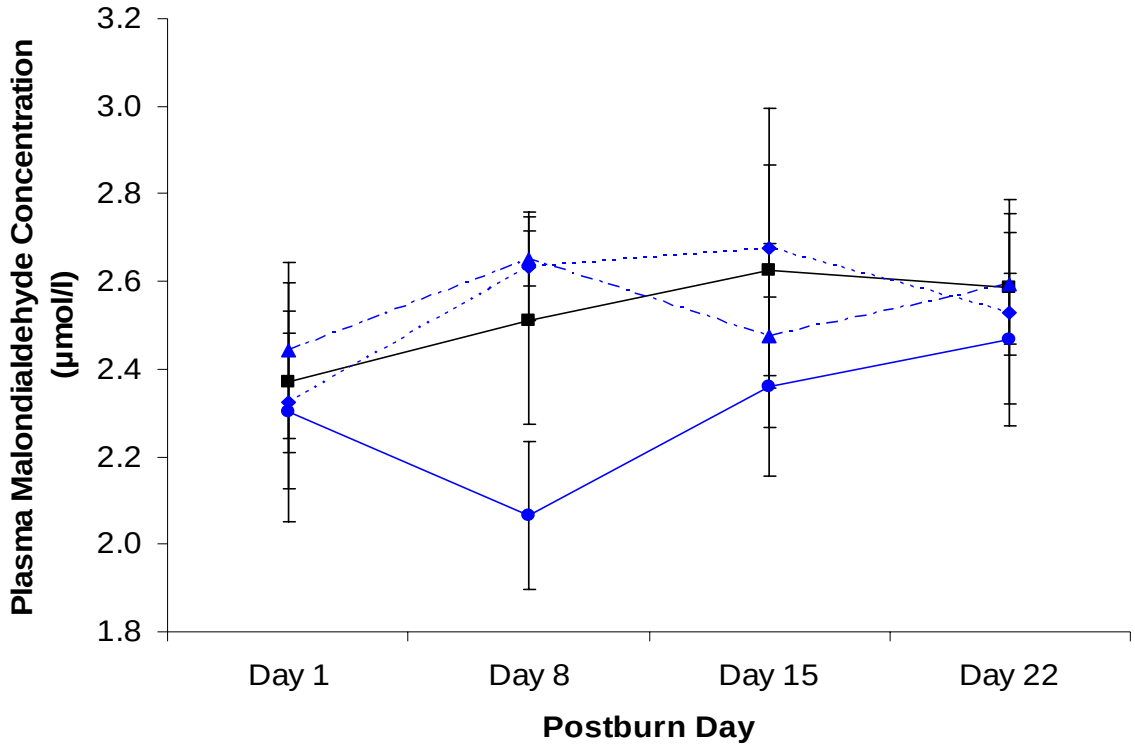

Figure 5-16. The plasma malondialdehyde concentrations of the **UNBURN group** rats after intake of **NS** (◆, dot), **GEL** (▲, semi-solid) and **EOC** (●, solid). The **CONTROL group** (■, solid) is also shown as reference for the normal malondialdehyde concentration of rats.

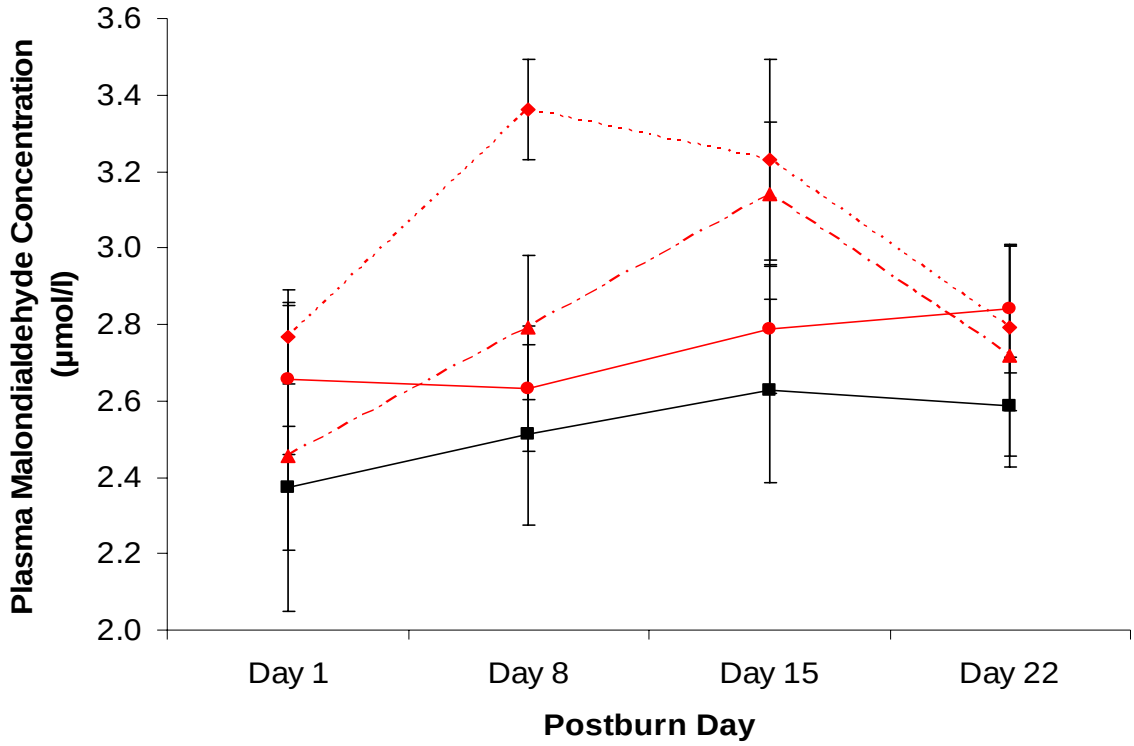

Figure 5-17. The plasma malondialdehyde concentrations of the **BURN group** rats after intake of **NS** (◆, dot), **GEL** (▲, semi-solid) and **EOC** (●, solid). The **CONTROL group** (■, solid) is also shown as reference for the normal plasma malondialdehyde concentration of rats.

5.4 Effects of Essence of Chicken on Blood Glucose Concentration in Normal and Burned Rats

The consumption of both GEL and EOC caused no significant effect in the UNBURN group in the experiment (Figure 5-18). On the other hand, the BURN-GEL showed a significantly high blood glucose level on day 22 postburn ($p=0.019$ vs. BURN-NS). A significantly high blood glucose level was also noticed for the BURN-EOC group on days 1 and 22 postburn ($p=0.045$ and $p=0.019$ vs. BURN-NS respectively) (Figure 5-19).

Table 5-6. The effects of NS, GEL and EOC on blood glucose concentrations in the UNBURN and BURN groups on days 1, 8, 15 and 22. CONTROL shows the normal blood glucose concentration.

	CONTROL	UNBURN			BURN		
	(n=6)	NS (n=6)	GEL (n=6)	EOC (n=6)	NS (n=6)	GEL (n=6)	EOC (n=6)
Blood Glucose (mmol/l)							
Day 1	10.24±0.25	10.53±0.49	10.62±0.67	11.04±0.66	9.12±0.67	9.69±0.79	11.10±0.95 *
Day 8	10.33±0.54	10.98±0.15	10.82±0.35	11.14±0.48	10.52±0.56	10.57±0.18	11.16±0.45
Day 15	10.60±0.18	10.72±0.40	10.98±0.30	11.01±0.40	10.57±0.47	11.25±0.37	10.93±0.36
Day 22	10.29±0.35	10.13±0.16	10.85±0.50	10.57±0.29	9.86±0.27	10.88±0.09 *	10.88±0.22 *

· The blood glucose concentrations are expressed as mean ± S.E.M. (*) indicates that the blood glucose concentration in this group was significantly lower than in the corresponding NS group ($p<0.05$). 6 rats were sacrificed at each specified time interval.

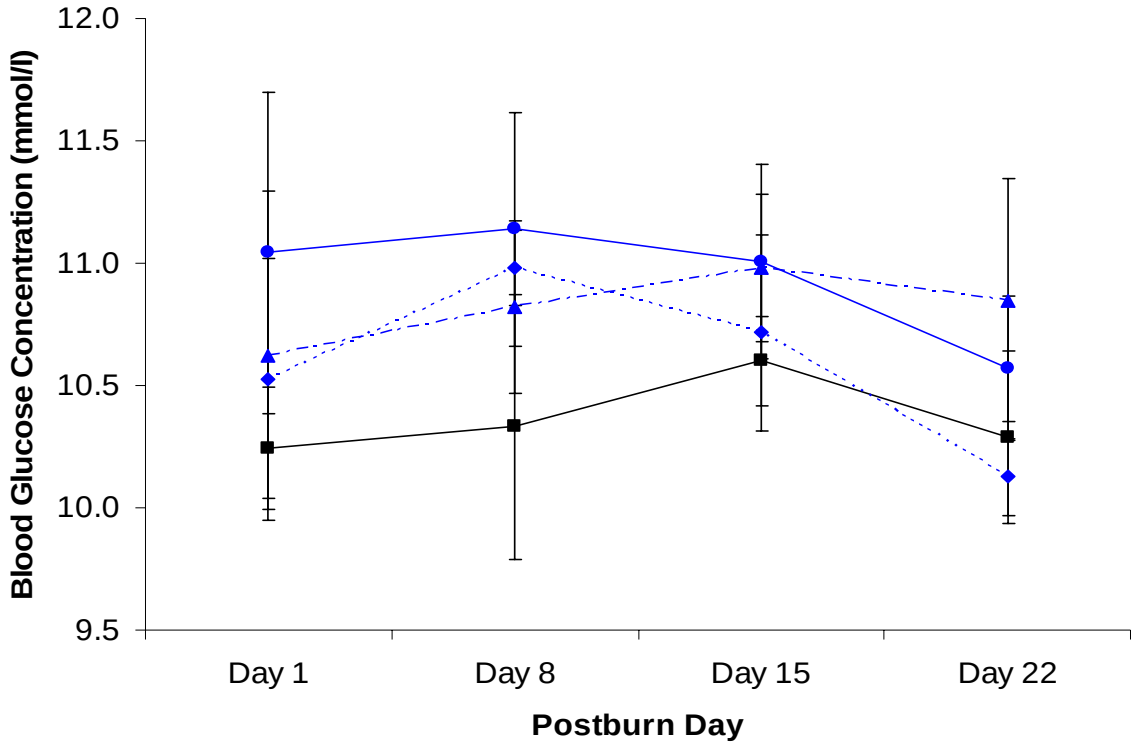

Figure 5-18. The blood glucose concentrations of the **UNBURN group** rats after intake of **NS** (◆, dot), **GEL** (▲, semi-solid) and **EOC** (●, solid). The **CONTROL group** (■, solid) is also shown as reference for the normal blood glucose concentration of rats.

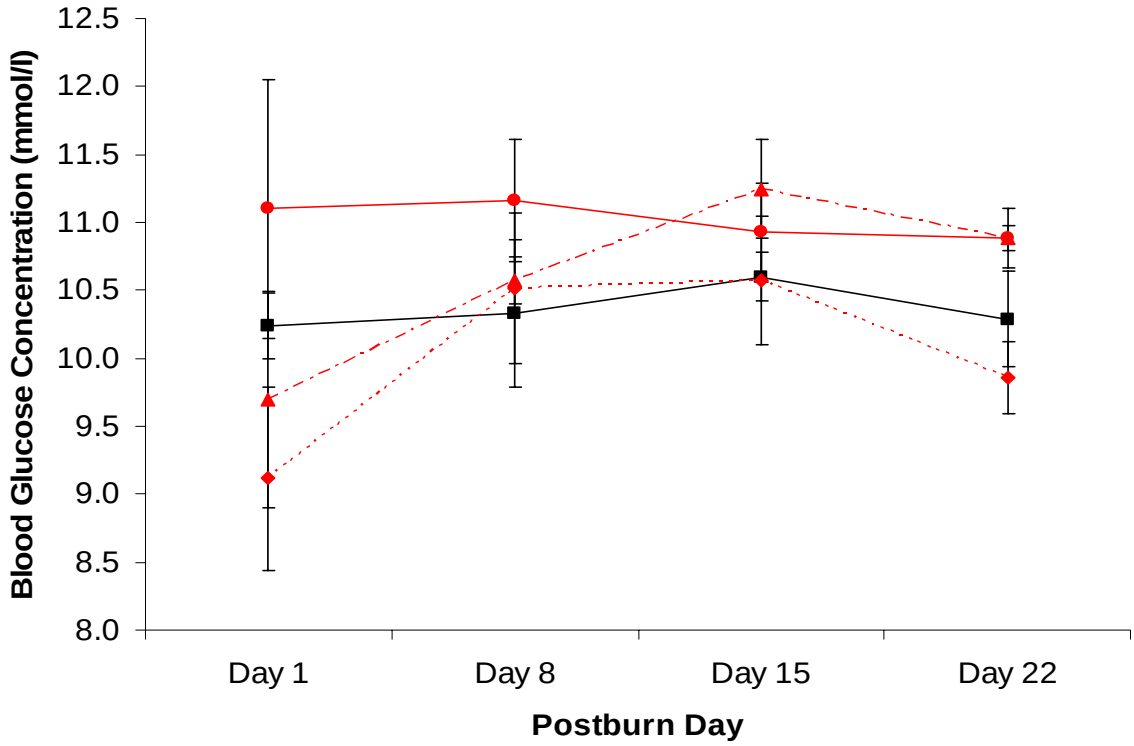

Figure 5-19. The blood glucose concentrations of the **BURN group** rats after intake of **NS** (◆, dot), **GEL** (▲, semi-solid) and **EOC** (●, solid). The **CONTROL group** (■, solid) is also shown as reference for the normal blood glucose concentration of rats.

5.5 Conclusion

This chapter reported the results of changes in serum IgG, IgA and IgM concentrations, plasma NO and MDA (marker of LPO) levels and blood glucose concentrations after the consumption of GEL and EOC. The changes were compared with their corresponding placebo NS group. In summary, we can conclude that the consumption of GEL and EOC increased the serum IgG, IgA and IgM concentrations in both the UNBURN and BURN groups on some days postburn. Further, both GEL and EOC showed inhibiting effects on NO and LPO in the BURN group, while only EOC was able to decrease the LPO level in the UNBURN group. Finally, both GEL and EOC were able to increase the blood glucose concentration of the BURN group, but no effect was found in the UNBURN group.

CHAPTER 6

DISCUSSION

6.1 Introduction

This chapter will explain the findings obtained in the laboratory work. The discussions will focus on the compatibility of our burn model with reference to the existing literature, differences in efficacy and constituents between GEL and EOC, alterations of serum immunoglobulin concentrations, plasma NO and LPO levels and blood glucose levels, and their possible benefits in term of the components in GEL and EOC. Finally, the implications of the effects on health will also be discussed.

6.2 Effects of Essence of Chicken on Immunological Responses in Normal and Burned Rats

To recapitulate, with regard to immunosuppression after burn injury, serum IgG, IgA and IgM decrease initially on day 1 postburn and reached a trough on around days 3-5 (Arturson *et al.*, 1969; Bariar *et al.*, 1994; Bjornson *et al.*, 1976; Munster *et al.*, 1970; Ninnemann, 1982; Sengupta *et al.*, 1980; Winkeldtein, 1984). IgG has usually shown a more profound depression than IgA and IgM (Hansbrough *et al.*, 1987; Kohn and Cort, 1969). Depending on the type of immunoglobulin, their levels return to normal gradually at different times within 1-2 months.

Burn injury resulted in similar consequences for the postburn immunoglobulin concentrations in this study. For the BURN groups, serum immunoglobulin (IgG,

IgA and IgM) concentrations decreased immediately after the burn infliction. This suggests that the burn trauma impaired the immune functions of the animals and initiated postburn immunosuppression. For the UNBURN group, the consumption of GEL resulted in an immediate serum IgG enhancement on day 1, while the effect on the EOC group came on day 8 postburn. On the other hand, both GEL and EOC elevated the serum IgG level in the BURN group on day 8. It seems that both GEL and EOC have the potential to enhance the serum IgG level for normal and scalded rats in the early stages. Figures 5-8 and 5-9 support these conclusions. Both figures clearly show that the serum IgG levels of the normal and scalded rats elevated faster after intake of GEL and EOC when compared to the rats that were fed with a placebo (NS) on days 1 to 8.

A significant increase of serum IgA level was found in the UNBURN group on day 15 after the consumption of EOC, while GEL appeared to have same effect on the normal rats. An interesting finding was noted in the results of the BURN group. A significantly low serum IgA level was found on days 1 and 15 after the consumption of GEL, which then elevated on day 22. On the other hand, the consumption of EOC for the scalded rats showed a significantly high serum IgA level on day 22 and there was no difference when compared with the corresponding NS group from day 1 to day 15. Hence, we demonstrated that EOC has better enhancement effects on serum IgA levels in scalded animals at the early postburn stage than GEL. GEL could not relieve the suppression of serum IgA levels, particularly in the early postburn stages. However, with reference to Figure 5-11, both GEL and EOC elevated the serum IgA concentrations of the burned rats at the end of the study. The serum IgA levels almost reached normal values. Therefore, both GEL and EOC are effective in increasing

serum IgA concentrations in burned rats in the late stages. Meanwhile, EOC revealed an up-regulatory effect on serum IgA level in normal rats on day 15. According to Figure 5-10, GEL also caused an elevation of serum IgA level in normal rats, but it was not statistically significant.

Unlike IgG and IgM, GEL and EOC caused relatively few significant effects on elevation of serum IgA levels throughout the experiment. This lesser effect on IgA may be due to its presence mainly in the mucous membranes and to the fact that it may be released into the circulation system only in small amounts. Therefore, the changes in serum IgA levels caused by burn injury and consumption of EOC were insignificant and difficult to detect.

For GEL, there was a significant increase in the serum IgM levels of the normal rats on day 22. EOC showed a boost-up effect on the serum IgM levels on days 1 and 22 as well. Therefore, GEL seemed to elevate the serum IgM levels in the normal rats after prolonged consumption, while EOC had a similar effect in the early and late stages of consumption. In the scalded animals, only EOC showed outstanding performance in elevating serum IgM levels on day 15. It is likely that only EOC has an up-regulatory effect on serum IgM for scalded rats, while GEL has no such effect. According to Figure 5-13, the consumption of EOC pulled up the serum IgM levels of the burned rats steadily throughout the experiment; GEL also showed a similar effect at the beginning, but was less efficacious than EOC. On the other hand, the consumption of EOC increased the serum IgM levels in the normal rats in the later stages of the experiment.

Interestingly, most of the EOC effects on serum immunoglobulin levels commenced on or after the 8th day postburn. Significant elevations were noticed in all immunoglobulin levels in both the UNBURN and BURN groups. This delay in serum immunoglobulin-promoting action is possibly due to the time needed for the digestion of the meat proteins and dipeptides of the EOC and the assimilation of additional immunoglobulin of the immune system.

When compared to controls, it seems that both GEL and EOC consumption are capable of increasing serum immunoglobulin concentrations in burned rats. Although immunoglobulin levels can be restored naturally after thermal injury, the recovery period may be extensive, and in a prolonged immunosuppressive state the incidence of post-injury morbidity and mortality may increase. Hence, a faster recovery of immunoglobulin levels after thermal injury has clinical significance. It may help to reduce susceptibility to bacterial and viral infections in patients with tissue injuries, such as surgery and trauma patients, and in turn may reduce morbidity and mortality. Nonetheless, EOC seems more efficacious in decreasing immunosuppression after burn injury than GEL, as EOC is more effective in increasing serum IgA and IgM levels. The increased immunoglobulin levels in normal rats reflect the ability of GEL and EOC consumption to enhance the immune functions of the body to meet the immunological challenges encountered in daily life. Conversely, the over-stimulated production of specific types of immunoglobulin, such as IgE, may sometimes be a detriment to health as they may provoke allergic reactions.

Both GEL and EOC are protein-rich substances. They may contain some proteins that have beneficial effects on immunological response. Sim (2002) reports that the

nutrients possessing these actions include amino acids (such as arginine, glutamine and glycine), fatty acids (such as omega-3 polyunsaturated fatty acids), antioxidants (such as vitamins C and E) and some micronutrients (such as copper, zinc and selenium). Saffle and Hildreth (2002) also point out that the provision of additional dietary intake can significantly improve the immune function and survival rate of burned children. Minehira *et al.* (2000) also found that immune function could be moderated by the selection and combination of dietary protein. However, EOC contains proteins, vitamins and nutrients, along with the known rich content of the active dipeptides carnosine and anserine, which support its potential to enhance immune function (Candlish, 1998; Hipkiss, 1998). This may explain why the immune-enhancing effects of GEL are less efficacious, as GEL contains proteins that are hydrolysed from fish collagen only. It does not contain the various immune-enhancing nutrients that are also required during the formation of immunocompetence. However, as the body needs time to assimilate the meat proteins, EOC should be consumed long-term to allow the immune-enhancing properties to take effect.

6.3 Effects of Essence of Chicken on Free Radical Scavenging in Normal and Burned Rats

In the present study, burn injury caused an increase in NO and LPO levels in the BURN group rats on day 1, and then stayed at high levels until the end of the study. For the NO study, the metabolic products of NO, NO_2^- and NO_3^- were used to indicate the NO concentrations in plasma. No significant difference was found between the UNBURN-GEL and EOC groups when compared to the corresponding NS group in the experiment. It seems that neither the consumption of GEL nor that of EOC caused

an effect on the plasma NO level of the normal rats. GEL and EOC revealed no disturbance to normal plasma NO levels, which are vital to health because NO is the modulator of vasodilation in the peripheral circulation system and hyperpolarization in the endothelial cells, whilst it also prevents the adherence of platelets and neutrophils in the microvasculature and protects cell functions (Horton, 2003; Moncada and Higgs, 1991; Vanhoutte, 2004). Therefore, NO should be maintained close to the normal level to ensure that the above mechanisms work properly. For the BURN group, the consumption of both GEL and EOC resulted in a significant depression of plasma NO level on day 15 postburn when compared to the respective BURN-NS group. These results confirm that both GEL and EOC possibly can promote the free radical scavenging effects on the excess NO of burned rats.

Although only one significant drop was found in the plasma NO level of the burned rats, Figure 5-15 shows that GEL and EOC commenced their effects on lowering the plasma NO levels of the burned rats on day 1 postburn. The plasma NO levels of both groups then decreased steadily from day 1 to day 22, and they were maintained at relatively low levels when compared to the BURN-NS group. Despite the statistical insignificance of these results, they demonstrate that both GEL and EOC are capable of inhibiting the immediate rise of plasma NO levels after burn injury, whilst the consecutive consumption of GEL and EOC can further remove burn-related pathologic NO from the body. In contrast to GEL, EOC showed more efficacy than GEL in NO scavenging, as EOC kept the plasma NO level at a lower level than GEL throughout the experiment.

MDA, a stable end-product of LPO, was used to indicate the plasma LPO level. A significant drop of LPO level was noticed in the normal rats on day 8 after the consumption of EOC, while the GEL caused no effect on the plasma LPO levels in the normal rats (vs. the corresponding UNBURN-NS group). For the BURN group, the consumption of both GEL and EOC led to a significant decrease of plasma LPO levels on day 8 postburn. It is likely that EOC has down-regulating effects on the LPO levels of both normal and burned rats, whereas GEL produces such effects in burned rats only. Nevertheless, EOC showed a statistically significantly low LPO level in both normal and burned rats on day 8 only. According to Figures 5-16 and 5-17, the consumption of EOC inhibited the LPO level at relatively lower levels than in the respective NS and GEL groups in both normal and burned rats. The difference is that the effects commenced on day 1for the burned rats, but on day 8 for the normal rats. Meanwhile, GEL also possessed inhibiting effects on the plasma LPO level in the burned rats but not in the normal rats.

It is well documented that one of the active ingredients of EOC - carnosine, possesses antioxidative and free radical scavenging function (Hipkiss, 1998; Hipkiss, Brownson and Carrier, 2001). Past studies showed carnosine can inhibit low-density lipoprotein oxidation and arterial plaque formation in a in vitro experiment (Bogardus and Boissonneault, 2000) and increase the lifespan of human CD4+ T cell clones by reducing its DNA oxidative damage (Hyland *et al.*, 2000). Peng and Lin (2004) also pointed out that carnosine- and anserine-containing chicken essence has enhancing effects on the activities of antioxidant enzymes, such as Glutamic-oxalacetatic transaminase (GOT) and glutamic-pyruvic transaminase (GPT), and protecting liver

from oxidative damage in carbon tetrachloride-induced oxidative stress rat model. These evidences may explain why EOC may bear antioxidative effects.

The use of antioxidant therapies after burn injury has been introduced for thermal injury patients to relieve their oxidative stress. It is believed that antioxidants can reduce the free radical-mediated tissue and cellular damage by maintaining microvascular permeability, which reduces protein and fluid loss, prevents the development of sepsis and inflammation that may cause further tissue injury, and promotes the restoration of immunity to reduce the susceptibility to infection (Cetinkale, Senel and Bulan, 1999; Horton, 2003). The present study demonstrated that both GEL and EOC are capable of eliminating or inhibiting burn-related NO and LPO in burned animals. Clinically, it is hoped that the consumption of GEL and EOC will help burned patients to remove excess NO and lipid peroxides postburn, and result in reduction of the incidence of free radical-mediated sepsis, MODS and inflammatory response that may contribute to the complications of burn injury. Further, GEL and EOC may be able to prevent the free radical-mediated immune dysfunction of burned patients, and hence may help to reduce both their susceptibility to infection and ultimately the incidence of NO- and LPO-related diseases and postburn morbidity and mortality.

Although both GEL and EOC demonstrate free radical scavenging effects on NO and LPO, EOC is preferable to GEL for patients as it is more efficacious than GEL, therefore it may immediately show superior efficacy for patients and help to promote early recovery from traumatic injury.

For the normal rats, EOC also showed the ability to inhibit LPO levels. Hence, the consumption of EOC has the potential to defer or diminish the occurrence of LPO-mediated diseases and ageing.

The presence of nourishing and special nutrients in EOC may contribute to its free radical scavenging effects. Many dietary nutrients bear antioxidative effects that can scavenge free radicals in the body. These natural free radical scavengers include catalase, SOD, glutathione peroxidase, uric acid-reduced glutathione, vitamin C and vitamin E (Cetinkale *et al.*, 1997; Nguyen *et al.*, 1993). EOC may contain some of these nutrients and may have such free radical scavenging effects. Moreover, one study suggested that EOC is rich in dipeptides from chicken meat, particularly carnosine and anserine (Candlish, 1998). Carnosine is a well-known antioxidant that has been proven effective in preventing the production of lipid peroxidation products, free radical scavenging, lowering T cells DNA damage, and even anti-ageing (Hipkiss, 1998; Hipkiss, Brownson and Carrier, 2001; Hyland *et al.*, 2000; Wu, Chen and Shiau, 2003). Apart from carnosine, anserine has also been found useful in preventing lipid peroxidation (Wu *et al.*, 2003). Because of the free radical scavenging of these constituents, it is possible that the consumption of EOC promotes health status by removing harmful free radicals from the body.

6.4 Effects of Essence of Chicken on Blood Glucose Concentration in Normal and Burned Rats

The results demonstrate that the consumption of GEL and EOC did not influence blood glucose levels in the normal rats. The underlying benefit is mostly due to the

hormonal blood glucose regulation system. As the system worked in fully functioning in normal rats, the glucose from the digested nutrients was utilized or stored by the regulation system according to blood glucose concentrations in the bloodstream. Therefore, there was no significant effect after the intake of GEL and EOC when compared to the corresponding NS group in the normal rats.

On the other hand, burn injury caused a drop in blood glucose levels in the burned rats on day 1 postburn. The consumption of GEL had no significant effect on blood glucose levels within the early postburn stage (from day 1 to day 15). Then, a significant high in blood glucose levels was recorded on day 22 postburn. Meanwhile, EOC consumption resulted in significantly high blood glucose levels on days 1 and 22 after thermal injury. With reference to Figures 5-18 and 5-19, the consumptions of EOC in both normal and burned rats showed relatively stable blood glucose levels throughout the experiment period. Therefore it is suggested that the consumption of EOC may help to stabilize blood glucose levels in both normal and burned rats, whereas GEL shows no such efficacy.

Because thermal injury can enhance the whole-body uptake of glucose for NIMGU and maintains the immune responses in some organs and tissues against infection (e.g. the lung, liver and spleen, and wounds) (Mizock, 2001), a steady and sufficient but not excessive blood glucose supply is suggested for patients after thermal or traumatic injury.

Clinically, the stabilization of blood glucose levels after the consumption of EOC may contribute to the normalization of frustrating elevations in blood glucose levels in

patients with thermal or traumatic injury. According to past studies, sustained hyperglycaemic status possibly leads to osmotic diuresis with hypovolaemia, electrolyte abnormalities and hyperosmolar non-ketotic coma (Mizock, 2001). Increases in blood glucose level may also be associated with catabolism in skeletal muscle and the depression of immune function, which result in exacerbation of post-injury infection complications and impairment of phagocytosis (Flakoll, Hill and Abumrad, 1993; Hill *et al.*, 1998; Saeed and Castle, 1998). The predicted consequences of EOC consumption prevent the occurrence of burn-related hyperglycaemia and related damage, whilst EOC can provide a stable and adequate blood glucose supply to maintain organ functions and support the recovery of wound and immune functions.

The findings of Yamano *et al.*'s study also suggest that the blood glucose regulatory effects of EOC may be ascribed to its major dipeptide component, L-carnosine. The study pointed out that L-carnosine is a possible endogenous control factor of blood glucose level through the modulation of the autonomic nerves via H3-receptor.

6.5 Conclusion

In conclusion, prolonged consumption of EOC revealed enhancing effects on serum IgG, IgA and IgM in normal and burned rats. Meanwhile, EOC also showed scavenging effects on excess NO in burned rats and LPO in both normal and burned rats. For blood glucose concentration, the consumption of EOC showed regulatory effects in both normal and burned rats, which in turn provided a stable and relatively high glucose supply to the rats and may have been beneficial to wound healing. EOC is rich in dipeptides and vitamins. Some of them may play roles in the effects

mentioned above, while some roles are still to be discovered. Hence, follow-up studies should be conducted to further explore the effects of EOC on health.

GEL also showed effects similar to EOC in enhancing immunoglobulin concentrations, lowering NO and LPO levels and modulating blood glucose level. However, its effects are far less substantial than those of EOC, as it is nothing more than a protein-rich substance.

CHAPTER 7

CONCLUSIONS

7.1 Introduction

This chapter will summarize all the effects of EOC on immunological response, free radical scavenging and blood glucose level. We will start by recapitulating the research objectives, then summarize the findings as well as the overall implications of the results and the limitations of the study. Suggestions will also be made with regard to further exploration on this topic.

7.2 Revisiting Research Objectives

Studies suggest that EOC contains some components that are capable of activating immune cells, scavenging free radicals in the autooxidative system and modulating blood glucose levels. Hence, the goals of this study were as follows:

1. To determine the effects of EOC on the concentration of serum IgG, IgA and IgM in normal and burned rats.

2. To examine whether EOC has any effect on free radical scavenging capacity.

3. To determine the effects of EOC on the concentration of serum glucose.

7.3 Summary of Findings

In this study, the following findings were observed:

1. EOC appeared to have enhancing effects on serum IgG, IgA and IgM in normal and burned rats. Prolonged consumption of EOC is required for its efficacious effects to commence.

2. EOC exhibited inhibiting effects on plasma NO level in burned rats and plasma LPO level in both normal and burned rats. Such effects are possibly due to EOC contains antioxidative dipeptides - carnosine and anserine, or it can promote the effects of antioxidants in body.

3. EOC showed stabilizing effects on blood glucose concentration in normal and burned rats.

4. GEL also demonstrated similar effects on up-regulation of immunoglobulin concentrations and removal of free radicals. Its efficacy was lower than that of EOC as it contains pure proteins only.

7.4 Implications

This study suggests that EOC is capable of increasing the serum IgG, IgA and IgM concentrations in normal and burned rats. This implies that EOC has the potential to enhance the immunological responses of both normal individuals and patients with traumatic injury. The enhancement of immune function in normal persons could help

to increase resistance to illness and reduce susceptibility to various kinds of viral and bacterial infections in daily life. The up-regulation of immunological responses after a burn or other traumatic injury is also vital to reduce the morbidity and mortality of the patient by preventing the incidence of sepsis and MODS, which are related to immune dysfunction. Therefore, EOC is recommended for both normal persons and individuals with an illness, particularly post-operative patients and patients with traumatic injury.

In addition to increasing immunological responses, EOC also has demonstrated scavenging effects on plasma NO in burned rats and plasma LPO in normal and burned rats. NO is produced by the constitutive NO synthases and takes part in various cell functions such as vasodilation modulation and hyperpolarization in the peripheral circulation system and endothelial cells respectively, prevents the adherence of platelets and neutrophils in microvasculature and protects cell functions (Horton, 2003; Moncada and Higgs, 1991; Vanhoutte, 2004). Hence, the consumption of EOC does not seem to disturb normal NO levels in the body. Meanwhile, EOC possibly removes excess NO production by iNOS after burn injury. On the other hand, EOC also showed inhibitory effects on LPO in normal individuals and persons with traumatic injury. All in all, the consumption of EOC has the potential to lower pathogenic NO and LPO levels, and it appears to contribute to the prevention of oxidative stress related to tissue damage. Hence, it is believed that the consumption of EOC may help to defer or diminish free radical-mediated diseases and even ageing, particularly for persons recovering from traumatic injuries.

In the last part of the study, EOC revealed stabilizing effects on blood glucose levels in normal and burned rats. The stabilization of blood glucose concentrations in normal individuals can sustain the organs of the body, which deal with a heavy daily workload, and is especially vital to the organs that need a high and stable blood glucose supply, such as the brain. A stable blood glucose supply may also be beneficial to burned, traumatically injured or post-operative patients, as it can prevent trauma-related hyperglycaemia and provide energy for tissue reparation and the maintenance of immune function.

7.5 Limitations

According to previous studies, the immune functions were affected most severely on the first 1-5 days postburn. This study has only determined changes in immunoglobulin levels on days 1 and 8 postburn, therefore information regarding changes between days 2 and 6 is missing, and the entire picture of the effects of EOC on early immunoglobulin level changes has not been shown. This problem is also present in studies of free radicals and blood glucose, as these biological parameters also change rapidly within the first few days postburn.

Moreover, the dose-response relationships between EOC and immunoglobulin concentrations, free radical levels and blood glucose concentrations were not known, as a fixed dosage was used in this study. Therefore, we have no idea what is the optimal dosage to maximize the effects of EOC. Besides, the effects of EOC on allergy-causing immunoglobulin IgE are still unknown, therefore, it is important to know whether EOC can lead to allergic reactions in consumers.

As the results are quite sporadic and no obvious trend can be obtained in all outcome parameters, It is also suggested to increase the sample size in order to enhance the difference due to EOC's effects.

7.6 Future Directions

The effects of EOC on the immune system and other immune components, such as T and B lymphocytes, interleukins and cluster designation systems (CDS), are still unclear. Similarly, apart from NO and LPO, the effects of EOC on other free radicals, such as superoxide and hydroxyl free radicals, and the effects of EOC on oxidative damage, including protein carbonyl and DNA damage, were not studied. They also play roles in increasing the oxidative stress of the body, and further studies are needed to complete the story.

Lipid peroxidation occurs commonly in many organs after burn injury. It is also suggested that investigate the levels of lipid peroxidation products (TBARS) in organs, such as liver, kidney and brain.

In addition, the optimal dosage and timing of EOC consumption in order to maximize its effects is a valuable avenue for future investigations. It is also unclear whether these effects would be observed in human subjects and their mechanisms. Again, these queries have to be supplemented by further studies.

7.7 Conclusion

This study was the first attempt to study the effects of EOC on immunological responses, free radical scavenging and blood glucose concentrations in vivo. It was found that EOC has positive effects that increase immunological responses in normal and burned rats. It also exhibited scavenging effects on plasma NO in burned rats and on plasma LPO level in normal and burned rats. Finally, a stabilizing effect was noticed on the blood glucose concentrations in normal and burned rats. This study serves to support further studies of EOC in humans on related aspects.

References

Abramson, S. B., Amin, A. R., Clancy, R. M. and Attur, M. (2001). The role of nitric oxide in tissue destruction. *Best Practice and Research Clinical Rheumatology*, 15(5), 831-845.

Arlt, S., Kontush, A., Zerr, I., Buhmann, C., Jacobi, C., Schröter, A., *et al.*, (2002). Increased lipid peroxidation in cerebrospinal fluid and plasma from patient with Creutzfeldt-Jakob disease. *Neurobiology of Disease*, 10(2), 150-156.

Arturson, G., Högman, C. F., Johansson, S. G. O. and Killander, J. (1969). Changes in immunoglobulin levels in severely burned patients. *Lancet*, 293(7594), 546-548.

Azhar, M. Z., Abdul Razak, K. and Mohsin, S. S. J. (2001). The use of chicken essence as an adjunct to psychotherapy in anxious subjects – A clinical assessment. *Malaysian Journal of Psychiatry*, 9(1), 13-22.

Barbato, J. E. and Tzeng, E. (2004). Nitric oxide and arterial disease. *Journal of Vascular Surgery*, 40(1), 187-193.

Bariar, L. M., Bal, A., Hasan, A. and Sharma, V. (1996). Serum levels of immunoglobulins in thermal burns. *Journal of the Indian Medical Association*, 94(4), 133-134.

Biffl, W. L., Moore, E. E. and Haenel, J. B. (2002). Nutrition support of the trauma patient. *Nutrition*, 18(11/12), 960-965.

Bjornson, A. B., Altemeier, W. A. and Bjornson, H. S. (1977). Changes in humoral components of host defense following burn trauma. *Annals of Surgery*, 186(1), 88-96.

Bogardus, S. L. and Boissonneault, G. A. (2000). Carnosine inhibits in vitro low-density lipoprotein oxidation. *Nutrition Research*, 20(7), 967-976.

Borderie, D., Hilliquin, P., Hernvann, A., Kahan, A., Menkes, C. J. and Ekindjian, O. G. (1999). Nitric oxide synthase is expressed in the lymphomononuclear cells of synovial fluid in patients with rheumatoid arthritis. *Journal of Rheumatology*, 26(10), 2083-2088.

Brennan, P. A., Thomas, G. J. and Langdon, J. D. (2003). The role of nitric oxide in oral diseases. *Archives of Oral Biology*, 48(2), 93-100.

Candlish J. K. (1998). A traditional blood remedy as a modulator of the respiratory burst of the human neutrophil: an in vitro study. *International Journal of Food Sciences and Nutrition*, 49(1), 55-63.

Cetinkale, O., Belce, A., Konukoglu, D., Senyuva, C., Gumustas, M. K. and Tas, T. (1997). Evaluation of lipid peroxidation and total antioxidant status in plasma of rats following thermal injury. *Burns*, 23(2), 114-116.

Cetinkale, O., Konukoğlu, D., Şenel, O., Kemerli, G. D. and Yazar, S. (1999). Modulating the functions of neutrophils and lipid peroxidation by FK506 in a rat model of thermal injury. *Burns*, 25(2), 105-112.

Centinkale, O., Senel, O. and Bulan, R. (1999). The effect of antioxidant therapy on cell-mediated immunity following burn injury in an animal model. *Burns*, 25(2), 113-118.

Chan, M. M. and Mattiacci, J. A. (2001). Nitric oxide: actions and roles in arthritis and diabetes. *The Foot*, 11(1), 45-51.

Chandra, R. K. and Kumari, S. (1994). Effects of nutrition on the immune system. *Nutrition*, 10(3), 207-210.

Chao, J. C.-J., Tseng, H.-P., Chang, C. W., Chien, Y.-Y., Au, H. K., Chen, J.-R., *et al.* (2004). Chicken extract affects colostrum protein compositions in lactating women. *Journal of Nutritional Biochemistry*, 15(1), 37-44.

Chui, S. H., Wan, K. C., Lam, C. W. K. and Lewis, W. H. P. (1989). Enzyme-linked immunosorbent assay for free thyroxin in human serum. *Clinical Chemistry*, 35(8), 1770-1772.

Cook, D. (2002). Classification of burn trauma. In C. B. Bousfield (Eds.), *Burn Trauma: Management and Nursing Care* (pp.13-29). London: Whurr Publishers Limited.

Danilov, A. I., Andersson, M., Bavand, N., Wiklund, N. P., Olsson, T. and Brundin, L. (2003). Nitric oxide metabolite determinations reveal continuous inflammation in multiple sclerosis. *Journal of Neuroimmunology*, 136(1-2), 112-118.

De Zwart, L. L., Meerman, J. H. N., Commandeur, J. N. M. and Vermeulen, N. P. E. (1999). Biomarkers of free radical damage applications in experimental animals and in humans. *Free Radical Biology and Medicine*, 26(1/2), 202-226.

Demling, R. H. and LaLonde, C. (1990a). Systemic lipid peroxidation and inflammation induced by thermal injury persists into the post-resuscitation period. *Journal of Trauma-Injury Infection and Critical Care*, 30(1), 69-74.

Demling, R. H. and LaLonde, C. (1990b). Early postburn lipid peroxidation: effect of ibuprofen and allopurinol. *Surgery*, 107(1), 85-93.

Eales, L.-J. (1997). *Immunology for life scientists: A basic introduction.* Chichester: John Wiley & Sons, Limited.

Esaki, T., Hayashi, T., Muto, T., Kano, H., Kumar, T. N., Asai, Y., *et al.* (2000). Expression of inducible nitric oxide synthase and Fas/Fas ligand correlates with the incidence of apoptotic cell death in atheromatous plaques of human coronary arteries. *Nitric Oxide*, 4(6), 561-571.

Emerit, J., Edeas, M. and Bricaire, F. (2004). Neurodegenerative diseases and oxidative stress. *Biomedicine and Pharmacotherapy*, 58(1), 39-46.

Fabbi, P., Ghigliotti, G., Brunelli, C., Balbi, M., Spallarossa, P., Rossettin, P., *et al.* (2003). Intense lipid peroxidation in premature clinical coronary atherosclerosis is associated with metabolic abnormalities. *Journal of Laboratory and Clinical Medicine*, 143(2), 99-105.

Fernández-Vizarra, P., Fernández, A. P., Castro-Blanco, S., Encinas, J. M., Serrano, J., Bentura, M. L., *et al.* (2004). Expression of nitric oxide system in clinically evaluated cases of Alzheimer's disease. *Neurobiology of Disease*, 15(2), 287-305.

Flakoll, P. J., Hill, J. O. and Abumrad, N. N. (1993). Acute hyperglycemia enhances proteolysis in normal man. *American Journal of Physiology*, 265(5), E715-E721.

Geissler, C., Boroumand-Naini, M., Harada, M., Iino, T., Hirai, K., Suwa, Y., *et al.* (1996). Chicken extract stimulates haemoglobin restoration in iron deficient rats. *International Journal of Food Sciences and Nutrition*, 47(4), 351-360.

Geissler, C., Boroumand-Naini, M. and Tomassen, C. (1989). Large acute thermic response to chicken essence in humans. *Nutrition Reports International*, 39(3), 547-556.

Godard, C., Bustos, M., Muñoz, M. and Nusslé, D. (1989). Value of a chicken-based formula for refeeding of chicken with protracted diarrhea and malnutrition in a developing country. *Journal of Pediatric Gastroenterology and Nutrition*, 9, 473-480.

Graham, A., Hogg, N., Kalyanaraman, B., O'Leary, V., Darley-Usmar, V. and Moncada, S. (1993). Peroxynitrite modification of –low-density lipoprotein leads to recognition by the macrophage scavenger receptor. *FEBS Letters*, 330(2), 181-185.

Gutteridge, J. M. C. (1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clinical Chemistry*, 41(12), 1819-1828.

Halliwell, B. and Gutteridge, J. M. C. (1989). *Free Radicals in Biology and Medicine*. New York: Oxford University Press.

Halliwell, B., Gutteridge, J. M. C. and Cross, C. E. (1992). Free radicals, antioxidants, and human disease: Where are we now? *Journal of Laboratory and Clinical Medicine*, 119(6), 598-620.

Handa, S., Mungrue, I. N., You, X.-M., Stewart, D. J. and Husain, M. (2004). Inducible nitric oxide synthase over-expression in arterial myocytes exacerbates neointima formation and atherosclerosis. *Cardiovascular Pathology*, 13(3, Suppl 1), S36.

Hansbrough, J. F., Zapata-Sirvent, R. L. and Peterson, V. M. (1987). Immunomodulation following burn injury. *Surgical Clinics of North America*, 67(1), 69-92.

Herndon, D. N. and Tompkins, R. G. (2004). Support of the metabolic response to burn injury. *Lancet*, 363(9424), 1895-1902.

Hershman, M. J., Cheadle, W. G., George C. D., Cost, K. M., Appel, S. H., Davidson, P. F., *et al.* (1988). The response of immunoglobulins to infection after thermal and nonthermal injury. *American Surgeon*, 54(7), 408-411.

Hill, J. R., Kown, G., Marshall, C. A. and McDaniel, M. L. (1998). Hyperglycemic levels of glucose inhibit interleukin 1 release from RAW 264.7 murine macrophages by activation of protein kinase C. *The Journal of Biological Chemistry*, 273(6), 3308-3313.

Hill, K. E., Zollinger, L. V., Watt, H. E., Carlson, N. G. and Rose, J. W. (2004). *Journal of Neuroimmunology*, 151(1-2), 171-179.

Hipkiss, A. R. (1998). Carnosine, a protective, anti-ageing peptide? *The International Journal of Biochemistry and Cell Biology*, 30(8), 863-868.

Hipkiss, A., R., Brownson, C. and Carrier, M. J. (2001). Carnosine, the anti-ageing, anti-oxidant dipeptide, may react with protein carbonyl groups. *Mechanisms of Ageing and Development*, 122(13), 1431-1445.

Hogg, N. and Kalyanaraman B. (1999). Nitric oxide and lipid peroxidation. *Biochimica et Biophysica Acta*, 1411 (2-3), 378-384.

Holm, C., Hörbrand, F., Mayr, M., Henckel von Donnersmarch, G. and Mühlbauer, W. (2004). Acute hyperglycaemia following thermal injury: friend or foe? *Resuscitation*, 60(1), 71-77.

Hoşnuter, M., Gürel, A., Babucçu, O., Armutcu, F., Kargi, E. and Işikdemir, A. (2004). The effect of CAPE on lipid peroxidation and nitric oxide levels in the plasma of rats following thermal injury. *Burns*, 30(2), 121-125.

Horton, J. W. (2003). Free radicals and lipid peroxidation mediated injury in burn trauma: the role of antioxidant therapy. *Toxicology*, 189(1-2), 75-88.

Huang, S.-C. and Kuo, J. C.-C. (2000). Concentrations and antioxidative activity of anserine and carnosine in poultry meat extracts treated with demineralization and papain. *Proceedings of the National Science Council, Republic of China – Part B, Life Sciences*, 24(4), 193-201.

Hyland, P., Duggan, O., Hipkiss, A., Barnett, C. and Barnett, Y. (2000). The effects of carnosine on oxidative DNA damage levels and in vitro lifespan in human peripheral blood derived CD4+ T cell clones. *Mechanisms of Ageing and Development*, 121(1-3), 203-215.

Ikeda, T., Nishijima, Y., Kiso, Y., Shibata, H., Ono, H. and Moritani T. (2001). Effects of chicken essence tablets on resting metabolic rate. *Bioscience, Biotechnology and Biochemistry*, 65(9), 2083-2086.

Jack C. de la Torre and Stefano, G. B. (2000). Impaired microvascular perfusion in Alzheimer's disease: role of constitutive nitric oxide. *Neurobiology of Ageing*, 21(Suppl 1), 84.

Jahoor, F., Desai, M., Herndon, D. N. and Wolfe, R. R. (1988). Dynamics of the protein metabolic response to burn injury. *Metabolism*, 37(4), 330-337.

Janeway, C. A., Travers, P., Walport, M. and Capra, J. D. (1999). *Immunobiology: The immune system in health and disease*. London: Current Biology Publications.

Kasapoglu, M. and Özben, T. (2001). Alterations of antioxidant enzymes and oxidative stress markers in aging. *Experimental Gerontology*, 36(2), 209-220.

Knott, C., Stern, G. and Wilkin, G. P. (2000). Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. *Molecular and Cellular Neurosciences*, 16(6), 724-739.

Kohn, J. and Cort, D. F. (1969). Immunoglobulins in burned patients. *Lancet*, 293(7599), 836-837.

Kubena, K. S. and McMurray, D. N. (1996). Nutrition and the immune system: A review of nutrient-nutrient interactions. *Journal of the American Dietetic Association*, 96(11), 1156-1164.

Kumar, R., Seth, R.K., Sekhon, M. S. and Bhargava, J. S. (1995). Serum lipid peroxide and other enzyme levels of patients suffering from thermal injury. *Burns*, 21(2), 96-97.

Leguit, P. Jr., Reerink-Brongers, E. and Eijsvoogel, V. P. (1973). Immunological studies in burn patients – II. Humoral immune response. *International Archives of Allergy and Applied Immunology*, 44(5), 706-716.

Lestaevel, P., Agay, D., Peinnequin, A., Cruz, C., Cespuglio, R., Clarençon, D., *et al.* (2003). Effects of a thermal injury on brain and blood nitric oxide (NO) content in the rat. *Burns*, 29(6), 557-562.

Lüth, H.-J., Münch, G. and Arendt, T. (2002). Aberrant expression of NOS isoforms in Alzheimer's disease is structurally related to nitrotyrosine formation. *Brain Research*, 953(1-2), 135-143.

Manuel do Rosário Caneira da Silva, Helder Mota Filipe, Rui Manuel Amaro Pinto, Maria Francisca Salaverría Timóteo de Carvalho, Manuel Maria Monteriro Godinho de Matos, Acácio Cordeiro Ferreira, *et al*. (1998). Nitric oxide and human thermal injury short term outcome. *Burns*, 24(3), 207-212.

Matsuda, T., Clark, N., Hariyani, G. D., Bryant, R. S., Hanumadass, M. L. and Kagan, R. J. (1987). The effect of burn wound size on resting energy expenditure. *Journal of Trauma-Injury Infection and Critical Care*, 27(2), 115-118.

Matsumura, Y., Kita, S., Ono, H., Kiso, Y. and Tanaka T. (2002). Preventive effect of a chicken extract on the development of hypertension in stroke-prone spontaneously hypertensive rats. *Bioscience, Biotechnology and Biochemistry*, 66(5), 1108-1110.

Matsumura, Y., Okui, T., Ono, H., Kiso, Y. and Tanaka, T. (2001). Antihypertensive effects of chicken extract against deoxycorticosterone acetate-salt-induced hypertension in rats. *Biological and Pharmaceutical Bulletin*, 24(10), 1181-1184.

Marnett, L. J. (2002). Oxy radicals, lipid peroxidation and DNA damage. *Toxicology*, 181-182(27), 219-222.

McCann, S. M., Licinio, J., Wong, M.-L., Yu, W. H., Karanth, S. and Rettorri, V. (1998). The nitric oxide hypothesis of aging. *Experimental Gerontology*, 33(7/8), 813-826.

Milner, E. A., Cioffi, W. G., Mason, A. D., McManus, W. F. and Pruitt, B. A. Jr. (1994). A longitudinal study of resting energy expenditure in thermally injured patients. *Journal of Trauma-Injury Infection and Critical Care*, 37(2), 167-170.

Minehira, K., Inoue, S., Nonaka, M., Osada, K., Yamada, K. and Sugano, M. (2000). Effects of dietary protein type on oxidized cholesterol-induced alteration in age-related modulation of lipid metabolism and indices of immune function in rats. *Biochimica et Biophysica Acta – Molecular and Cell Biology of Lipids*, 1483(1), 141-153.

Miquel, J., Ramirez-Boscá, A., Soler, A., Díez, A., Carrión-Gutiérrez, M. A., Díaz-Alperi, J., *et al.* (1998). Increase with age of serum lipid peroxides: Implications for the prevention of atherosclerosis. *Mechanisms of Ageing and Development*, 100(1), 17-24.

Mizock, B. A. (2001). Alterations in fuel metabolism in critical illness: hyperglycaemia. *Best Practice and Research Clinical Endocrinology and Metabolism*, 15(4), 533-551.

Moncada, S. and Higgs, E. A. (1991). Endogenous nitric oxide: physiology, pathology and clinical relevance. *European Journal of Clinical Investigation*, 21(4), 361-374.

Montine, K. S., Quinn, J. F., Zhang, J., Fessel, J. P., Jackson Roberts II, Morrow, J. D., *et al.* (2004). Isoprostanes and related products of lipid peroxidation in neurodegenerative diseases. *Chemistry and Physics of Lipids*, 128(1-2), 117-124.

Munster, A. M., Hoagland, H. C. and Pruitt. B. A. Jr. (1970). The effect of thermal injury on serum immunoglobulins. *Annals of Surgery*, 172(6), 965-969.

Nagai, H., Harada, M., Nakagawa, M., Tanaka, T., Gunadi, B., Justinus Setiabudi, M. L., *et al.* (1996). Effects of chicken extract on the recovery from fatigue caused by mental workload. *Applied Human Science*, 15(6), 281-286.

Nagane, N. S., Bhagwat, V. R. and Subramanium, M. (2003). Increased free radical activity in burns. *Indian Journal of Medical Sciences*, 57(1), 7-11.

Nazliel, B., Taşkiran, D., Irkec, C., Kutay, F. Z. and Pöğün, Ş. (2002). Serum nitric oxide metabolites in patients with multiple sclerosis. *Journal of Clinical Neuroscience*, 9(5), 530-532.

Nguyen, T. T., Cox, C. S., Traber, D. L., Gasser, H., Redl, H., Schlag, G., *et al.* (1993). Free radical activity and loss of plasma antioxidants, vitamin E, and sulfhydryl groups in patients with burns. *Journal of Burn Care and Rehabilitation*, 14(6), 602-609.

Ninnemann, J. L. (1982). Immunologic defenses against infection: Alterations following thermal injuries. *Journal of Burn Care and Rehabilitation*, 3(6), 355-366.

Ninnemann, J. L., Fisher, J. C. and Wachtel, T. L. (1980). Effect of thermal injury and subsequent therapy on serum protein concentrations. *Burns*, 6(3), 165-173.

Ochoa, J. B. and Ford, H. R. (2002). Invited commentary: New roles for immune-enhancing diets. *Surgery*, 132(5), 815-816.

Onuoha, G., Alpar, K. and Jones, I. (2001). Vasoactive intestinal peptide and nitric oxide in the acute phase following burns and trauma. *Burns*, 27(1). 17-21.

Oranje, W. A., Rondas-Colbers, G. J. W. M., Swennen, G. N. M. and Wolffenbuttel, B. H. R. (1998). Lipid peroxidation in type 2 diabetes: Relationship with microvascular disease? *The Netherlands Journal of Medicine*, 53(2), 61-68.

Ou, J. J., Zhang, Y. and Montine, T. J. (2002). In vivo assessment of lipid peroxidation products associated with age-related neurodegenerative diseases. *Experimental Neurology*, 175(2), 363-369.

Parks, D. A., Skinner, K. A., Skinner, H. B. and Tan S. (1998). Multiple organ dysfunction syndrome: Role of xanthine oxidase and nitric oxide. *Pathophysiology*, 5(1), 49-66.

Peck, M. D. and Chang, Y. (1999). Review: Nutritional support for burn injuries. *The Journal of Nutritional Biochemistry*, 10(7), 380-396.

Peng, H. C. and Lin, S. H. (2004). Effects of chicken extract on antioxidative status and liver protection under oxidative stress. *Journal of Nutritional Science and Vitaminology*, 50(5), 325-329.

Pintaudi, A. M., Tesoriere, L., D'Arpa, N., D'Amelio, L., D'Arpa, D., Bongiorno, A., *et al.* (2000). Oxidative stress after moderate to extensive burning in humans. *Free Radical Research*, 33(2), 139-146.

Punchard, N. A. and Kelly, F. J. (1996). Introduction. In N. A. Punchard and F. J. Kelly (Eds.), *Free Radicals: A Practical Approach* (pp. 1-8). New York: Oxford University Press.

Rawlingson, A. (2003). Nitric oxide, inflammation and acute burn injury. *Burns*, 29(7), 631-640.

Rennard, B. O., Ertl, R. F., Gossman, G. L., Robbins, R. A. and Rennard, S. I. (2000). Chicken soup inhibits neutrophil chemotaxis in vitro. *Chest*, 188(4), 1150-1157.

Riobó, N. A., Schöpfer, F. J., Boveris, A. D., Cadenas, E. and Poderoso, J. J. (2002). The reaction of nitric oxide with 6-hydroxydopamine: implications for Parkinson's disease. *Free Radical Biology and Medicine*, 32(2), 115-121.

Ritzmann, S. E., McClung, C., Falls, D., Larson, D. L., Abston, S. and Goldman, A. S. (1969). Immunoglobulin levels in burned patients. *Lancet*, 293(7606), 1152-1153.

Rolandelli, R. H., Koruda, M. J., Fukuchi, S. G., Rombeau, J. L. and Stein, T. P. (1998). The effect of glutamine- and alanine-enriched total parenteral nutrition on postburn proteolysis in the rat. *The Journal of Nutritional Biochemistry*, 9(1), 28-30.

Saeed, F. A. and Castle, G. E. (1998). Neutrophil chemiluminescence during phagocytosis is inhibited by abnormally elevated levels of acetoacetate: Implications for diabetic susceptibility to infections. *Clinical and Diagnostic Laboratory Immunology*, 5(5), 740-743.

Saffle, J. R. and Hildreth, M. (2002). Metabolic support of the burned patient. In D. Herndon (Eds.), *Total Burn Care* (pp. 271-287). London: Harcourt Publishers Limited.

Saitoh, D., Takasu, A., Fukuzuka, K., Norio, H., Sakamoto, T. and Okada, Y. (2001). Analysis of plasma nitrite/nitrate in human thermal injury. *The Tohoku Journal of Experimental Medicine*, 194(2), 129-136.

Schwacha, M. G. (2003). Macrophages and post-burn immune dysfunction. *Burns*, 29(1), 1-14.

Sengupta, S. R., Sukhtamkar, A. Y., Dhole, T. N. and Dubey, K. P. (1982). Humoral immunity in burns. *Burns*, 8(3), 172-175.

Sim, K. M. (2002). Management of severe burns injuries – a metabolic perspective. *Current Anaesthesia and Critical Care*, 13(2), 76-82.

Sim, M. K. (2001). Cardiovascular actions of chicken-meat extract in normo- and hypertensive rats. *British Journal of Nutrition*, 86(1), 97-103.

Smith, K. J. and Lassmann, H. (2002). The role of nitric oxide in multiple sclerosis. *Lancet Neurology*, 1(4), 232-241.

Spiteller, G. (2001). Lipid peroxidation in aging and age-dependent diseases. *Experimental Gerontology*, 36(9), 1425-1457.

Szabó, C. (2003). Multiple pathways of peroxynitrite cytotoxicity. *Toxicology Letters*, 140-141, 105-112.

Tinbergen, B. J. and Slump, P. (1976). The detection of chicken meat in meat products by means of the anserine/carnosine ratio. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, 161, 7-11.

Tsi, D., Khow, A., Iino, T., Kiso, Y. and Ono, H. (2003). Effect of Brand's glucosamine with essence of chicken on collagen-induced arthritis in rats. *Life Sciences*, 73(23), 2953-2962.

Vanhoutte, P. M. (2004). Endothelium-dependent hyperpolarizations: the history. *Pharmacological Research*, 49(6), 503-508.

Virág, L., Szabó, É., Gergely, P. and Szabó C. (2003). Peroxynitrite-induced cytotocicity: mechanism and opportunities for intervention. *Toxicology Letters*, 140-141, 113-124.

Waker, H. L. and Mason, A. D. (1968). A standard animal burn. *The Journal of Trauma*, 8(6), 1049-1051.

Warden, G. D. and Ninnemann, J. L. (1981). The immune consequences of thermal injury: An overview. In J. L. Ninnemann (Eds.), *The Immune Consequences of Thermal Injury* (pp. 1-20). Baltimore: The Williams and Wilkins Company.

Williams, A. T. and Schey, S. A. (1993). Use of traditional blood remedy. A study on regular blood donors. *International Journal of Food Sciences and Nutrition*, 44, 17-20.

Wilmore, D. W., Moylan, J. A., Pruitt, B. A., Lindsey, C. A., Faloona, G. R. and Unger, R. H. (1974). Hyperglucagonæmia after burns. *Lancet*, 303(7847), 73-75.

Wink, D. A. and Mitchell, J. B. (1998). Chemical biology of nitric oxide: Insights into the regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. *Free Radical Biology and Medicine*, 25(4/5), 434-456.

Winkelstein, A. (1984). What are the immunological alterations induced by burn injury? *The Journal of Trauma*, 24(9 Suppl.), S72-S83.

Witte, M. B. and Barbul, A. (2002). Role of nitric oxide in wound repair. *The American Journal of Surgery*, 183(4), 406-412.

Woolliscroft, J. O., Prasad, J. K., Thomson, P., Till, G. O. and Fox, I. H. (1990). Metabolic alterations in burn patients: detection of adenosine triphosphate degradation products and lipid peroxides. *Burns*, 16(2), 92-96.

Wu, H.-C., Chen, H.-M. and Shiau, C.-Y. (2003). Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Research International*, 36(9-10), 949-957.

Wu, H. C., Shiau, C. Y., Chen, H. M. and Chiou, T. K. (2003). Anti-oxidant activities of carnosine, anserine and some free amino acids and their synergistic effects in combination. *Journal of Food and Drug Analysis*, 11, 148-153.

Xu, C. L. and Sim, M. K. (1997). Effect of oral feeding of essence of chicken on the level of 5-hydroxyindole acetic acid in the cerebrospinal fluid of the rat. *International Journal of Food Sciences and Nutrition*, 48, 113-117.

Yamano, T., Niijima, A., Iimori, S., Tsuruoka, N., Kiso, Y. and Nagai, K. (2001). Effect of L-carnosine on the hyperglycemia caused by intracranial injection of 2-deoxy-D-glucose in rats. *Neuroscience Letters*, 313(1-2), 78-82.

Yokoyama, M. (2004). Oxidant stress and atherosclerosis. *Current Opinion in Pharmacology*, 4(2), 110-115.