THE HEP573 STUDY:

A randomised, double-blind, placebo-controlled clinical trial of silymarin alone, and silymarin combined with antioxidants in chronic hepatitis C

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A thesis submitted for the Degree of Doctor of Philosophy
School of Medicine and Public Health
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STATEMENT OF ORIGINALITY

The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University’s Digital Repository, subject to the provisions of the Copyright Act 1968.

STATEMENT OF COLLABORATION

Experts were consulted when necessary in the design and analysis stages as specified in the acknowledgments section and in Chapter 3 Methodology.

Acknowledgment of Authorship

The Study protocol design and coordination, analysis and reporting of the results were all undertaken by the author.

Date.
DEDICATION

This work is dedicated to the late Mr Robert John Salmond, my father, who taught me reverence for life, the art of perseverance and the wonder of humour. It is also dedicated to the loving memory of Ms Riwia Whaanga, Dr Lisa MacDonald, Ms Julie Velthuys and Mr Sam Richardson (who all died during the writing of this dissertation).

This also honours all those pioneers, ahead of their time in their respective fields, who willingly embraced other paradigms but, in so doing, needed to weather the attitude of their colleagues until a change in the dominant mindset prevailed.
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AWARD, PUBLICATIONS AND PRESENTATIONS FROM THIS STUDY

AWARD

Douglas Piper Young Investigator Award Clinical Science, 22 October 2010
Gastroenterological Society of Australia

RESEARCH PUBLICATIONS AND PRESENTATIONS

Publications

Peer-reviewed journal articles


Conference proceedings


Book chapters

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ALA/LA</td>
<td>alpha lipoic acid/lipoic acid/thioctic acid</td>
</tr>
<tr>
<td>ALD</td>
<td>alcoholic liver disease</td>
</tr>
<tr>
<td>ALT</td>
<td>alanine aminotransferase</td>
</tr>
<tr>
<td>AP-1</td>
<td>activator protein-1</td>
</tr>
<tr>
<td>ARE</td>
<td>antioxidant response element</td>
</tr>
<tr>
<td>AST</td>
<td>aspartate aminotransferase</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>CAM</td>
<td>complementary and alternative medicine</td>
</tr>
<tr>
<td>CAT</td>
<td>catalase</td>
</tr>
<tr>
<td>CHC</td>
<td>chronic hepatitis C</td>
</tr>
<tr>
<td>CLD</td>
<td>chronic liver disease</td>
</tr>
<tr>
<td>CM</td>
<td>complementary medicine</td>
</tr>
<tr>
<td>C of A</td>
<td>certificate of analysis</td>
</tr>
<tr>
<td>CTL</td>
<td>cytotoxic T lymphocyte</td>
</tr>
<tr>
<td>CR</td>
<td>calorie restriction</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>ECM</td>
<td>extracellular matrix</td>
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<tr>
<td>ERK</td>
<td>extracellular signal-regulated protein kinase</td>
</tr>
<tr>
<td>ESLD</td>
<td>end-stage liver disease</td>
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<tr>
<td>EVR</td>
<td>early virological response</td>
</tr>
<tr>
<td>F</td>
<td>fibrosis staging on liver biopsy</td>
</tr>
<tr>
<td>FM</td>
<td>fibrosis markers</td>
</tr>
<tr>
<td>GC-MS</td>
<td>gas chromatography coupled to mass spectrometry</td>
</tr>
<tr>
<td>GGT</td>
<td>gamma-glutamyltranspeptidase</td>
</tr>
<tr>
<td>GPx</td>
<td>glutathione peroxidase</td>
</tr>
<tr>
<td>GSH</td>
<td>glutathione (reduced)</td>
</tr>
<tr>
<td>GSSG</td>
<td>glutathione (oxidised)</td>
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<tr>
<td>GST</td>
<td>glutathione transferase</td>
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<tr>
<td>HA</td>
<td>hyaluronic acid</td>
</tr>
<tr>
<td>HCC</td>
<td>hepatocellular carcinoma</td>
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<tr>
<td>HCV</td>
<td>hepatitis C virus</td>
</tr>
<tr>
<td>HCV RNA</td>
<td>hepatitis C virus ribonucleic acid</td>
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<tr>
<td>HLA</td>
<td>human leukocyte antigen</td>
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<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
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<tr>
<td><strong>Abbreviation</strong></td>
<td><strong>Full Form</strong></td>
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<tr>
<td>HQLQ™</td>
<td>hepatitis quality of life questionnaire</td>
</tr>
<tr>
<td>HRQoL</td>
<td>health related quality of life</td>
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<tr>
<td>HSC</td>
<td>hepatic stellate cell</td>
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<tr>
<td>IFN</td>
<td>interferon</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>INN</td>
<td>International Nonproprietary Name</td>
</tr>
<tr>
<td>ISO</td>
<td>$F_2$-Isoprostanes</td>
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<tr>
<td>IVDU</td>
<td>intravenous drug use</td>
</tr>
<tr>
<td>KIR</td>
<td>killer cell immunoglobulin-like receptor</td>
</tr>
<tr>
<td>MCP</td>
<td>monocyte chemoattractant protein</td>
</tr>
<tr>
<td>MDA</td>
<td>malondialdehyde</td>
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<tr>
<td>MF</td>
<td>myofibroblast</td>
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<tr>
<td>MIP</td>
<td>macrophage inflammatory protein</td>
</tr>
<tr>
<td>NAC</td>
<td>N-acetyl cysteine</td>
</tr>
<tr>
<td>NADPH</td>
<td>nicotinamide adenine dinucleotide phosphate (reduced)</td>
</tr>
<tr>
<td>NAFLD</td>
<td>non-alcoholic fatty liver disease</td>
</tr>
<tr>
<td>NF-kB</td>
<td>nuclear factor kappa B</td>
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<tr>
<td>NK</td>
<td>natural killer cell</td>
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<tr>
<td>Nrf2</td>
<td>nuclear erythroid factor-2</td>
</tr>
<tr>
<td>NSW</td>
<td>New South Wales, an Australian State</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>OS</td>
<td>oxidative stress</td>
</tr>
<tr>
<td>P</td>
<td>placebo</td>
</tr>
<tr>
<td>$P$</td>
<td>probability value</td>
</tr>
<tr>
<td>P53</td>
<td>tumour protein 53</td>
</tr>
<tr>
<td>PCD</td>
<td>programmed cell death</td>
</tr>
<tr>
<td>PDGF</td>
<td>platelet-derived growth factor</td>
</tr>
<tr>
<td>PNAL</td>
<td>persistently normal ALT level</td>
</tr>
<tr>
<td>PPARγ</td>
<td>peroxisome proliferator-activated receptor gamma</td>
</tr>
<tr>
<td>RCT</td>
<td>randomised controlled trial</td>
</tr>
<tr>
<td>RDBPCT</td>
<td>randomised, double-blind, placebo-controlled trial</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
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<tr>
<td>RNS</td>
<td>reactive nitrogen species</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>RVR</td>
<td>rapid virological response</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
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</tr>
<tr>
<td>S</td>
<td>silymarin</td>
</tr>
<tr>
<td>SOD</td>
<td>superoxide dismutase</td>
</tr>
<tr>
<td>SOX</td>
<td>silymarin and antioxidant</td>
</tr>
<tr>
<td>STAT</td>
<td>signal transducer and activator of transcription</td>
</tr>
<tr>
<td>SVR</td>
<td>sustained virological response</td>
</tr>
<tr>
<td>TLC</td>
<td>thin-layer chromatography</td>
</tr>
<tr>
<td>TLRs</td>
<td>toll-like receptors</td>
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<td>TM</td>
<td>traditional medicine</td>
</tr>
<tr>
<td>TGF-β</td>
<td>transforming growth factor beta</td>
</tr>
<tr>
<td>TNF-α</td>
<td>tumour necrosis factor alpha</td>
</tr>
<tr>
<td>Treg</td>
<td>T regulatory cell</td>
</tr>
<tr>
<td>TRX, Trx</td>
<td>Thioredoxin</td>
</tr>
<tr>
<td>UV/VIS</td>
<td>ultra-violet and visible spectroscopy</td>
</tr>
<tr>
<td>WBC</td>
<td>white blood cell</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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GLOSSARY

Antioxidant is a substance that markedly slows or prevents oxidation of a substrate, when the substance is in low concentrations compared to that substrate.¹

Complementary medicine is an approach to health-care delivery that incorporates disease diagnosis, treatment and/or prevention and adds to conventional medicine by satisfying unmet demand or by broadening orthodox medicine’s theoretical structures.²

Compensated hepatitis C is an early phase of end-stage liver disease characterised by asymptomatic cirrhosis.³ It is reflected in a low level of complications from cirrhosis (e.g., jaundice, ascites, coagulopathy, and encephalopathy) as is characterised by a Child-Pugh Score of less than 7.

 Decompensated hepatitis C is the advanced phase of end-stage liver disease characterised by portal hypertension and or liver dysfunction³ (jaundice, ascites, or hepatic encephalopathy).⁴ The Child-Pugh Score is greater than 7.

Free radicals are molecules with an outer (valence) shell that contains an unpaired electron.⁵

International Nonproprietary Names (INN) help to identify pharmaceuticals or their active ingredients. Each INN provides a unique name that is public property and recognisable globally. A nonproprietary name is otherwise known as a generic name.⁶

Karyorrhexis is the fragmentation of the nucleus.⁷,⁸

Oxidative damage refers to the biomolecular harm when a reactive species attacks during oxidative stress.⁹

Oxidative stress (OS) is an imbalance between oxidants (reactive species production⁹, radical generating activity¹⁰) and antioxidants (antioxidant defence,⁹ radical scavenging activity¹⁰) in favour of the oxidants, potentially leading to (tissue) damage.⁵

Powdered extract (P.E.) refers to a dried extract. Fresh or dried plant material may be extracted in water, methanol, ethanol or other solvents to produce a liquid extract. This extract is then typically spray-dried to produce a dry or powdered extract. The powdered extract ratio is required for calculating the corresponding crude drug (plant) amounts e.g. P.E. 5:1 indicates that 5 kg of dried plant material was used to produce 1 kg of dried extract. The powdered extract ratio is synonymous with the drug-to-extract ratio.¹¹
Reactive oxygen species (ROS) is a collective term for oxygen radicals, such as superoxide anion (O\textsubscript{2}\textsuperscript{-}), hydroxyl radical (OH), hydroperoxyl (HO\textsubscript{2}), peroxyl (RO\textsubscript{2}), alkoxyl (RO) and carbon dioxide (CO\textsubscript{2}). It also includes some non radicals which are oxidising agents and/or are easily converted into radicals, such as hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), ozone (O\textsubscript{3}), singlet oxygen (O\textsubscript{2}^1), organic peroxides (ROOH), and peroxynitrite (ONOO).\textsuperscript{9}

Reactive nitrogen species (RNS) collectively refers to radicals of nitric oxide (NO) and nitrogen dioxide (NO\textsubscript{2}). It also includes some non radicals such as nitrous acid (HNO\textsubscript{2}), dinitrogen tetroxide (N\textsubscript{2}O\textsubscript{4}) and peroxynitrite (ONOO).\textsuperscript{9}

Redox status refers to the ratio of reduced (GSH) glutathione to oxidised (GSSG) glutathione.\textsuperscript{12}

Social determinants of health are conditions (including health systems) influenced by the distribution of resources, money and power locally, nationally and globally. These, in turn, are influenced by policy decisions. Social determinants of health are the main causes of health inequities within and between countries.\textsuperscript{13}

Standardisation means uniformity of all required manufacturing steps, from the crude drug to the final extract, in order to achieve a defined product standard (specification). Herbal extracts are typically standardised to a particular marker compound which may in some cases also be considered the active compound.\textsuperscript{11}

Traditional medicine comprises all knowledge, skills and practices which derive from indigenous ideas, beliefs and experiences of different cultures in order to maintain health, and to prevent, diagnose, treat, or improve physical and mental illnesses.\textsuperscript{14}
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PREFACE

Chapter 1 Introduction: outlines the scientific aims of the Study.

Chapter 2 Literature Review: contains an overview of the hepatitis C virus (HCV) infection; epidemiology, virology, natural history, causes of liver injury including viral, immune, oxidative stress (OS) and the pathobiology of the disease. It also examines the clinical implications of chronic HCV infection and the current management strategies for the chronic hepatitis C (CHC) patients within naturopathic and allopathic paradigms.

Chapter 3 Methodology: outlines the Study design, procedures, quality control, outcome measures and statistical analyses.

Chapter 4 Results: reports on the results achieved in the Hep573 Study.

Chapter 5 Discussion: presents the findings, strengths, limitations and implications of the Study and outlines future research directions.

Chapter 6 Conclusion: offers some concluding remarks.

The Appendices: include all approved, supporting documentation related to the conduct of the Study.

Throughout this dissertation, the research undertaken will be referred to as the ‘Hep573 Study’ or the ‘Study’.
ABSTRACT

Oxidative stress (OS) is a key mechanism by which liver injury occurs in chronic hepatitis C (CHC) virus infection. For this Study, it was hypothesised the use of antioxidant compounds would reduce OS, hepatic necroinflammation and hepatic fibrosis in CHC patients. To test this hypothesis, a randomised, double-blind, placebo-controlled clinical trial (termed the ‘Hep573 Study’) was conducted in three Australian teaching hospitals in New South Wales.

One hundred and eighteen participants were recruited through the liver outpatient clinics at the hospitals from July, 2003 to March, 2006. They were randomised to treatment in blocks of six to one of three groups: placebo; silymarin (720 mg silybin/day); and silymarin with antioxidants (720 mg silybin plus 13 other ingredients).

Study duration was 48 weeks: 24 weeks on active treatment or placebo, and 24 weeks follow-up post treatment.

The primary outcome measure was the proportion of patients with alanine aminotransferase (ALT) normalisation at Week 24 (Fisher’s exact test). Secondary outcome measures were the percentage change from baseline to Week 24 in F₂-isoprostanes, and to Week 24 and Week 48 in ALT, HCV viral load (HCV RNA) and FibroTest (Linear Mixed Effects). Results were analysed on an intention-to-treat basis.

In patients with compensated CHC, the use of silymarin and antioxidant compounds achieved a higher rate of ALT normalisation than placebo (P=0.02) or silymarin (P=0.003) at Week 24. This result could not be attributed to alcohol, diet or caffeine, as intake across the groups did not change throughout the Study. In addition, there was a significant improvement in the overall mental-health score (Mental Component Summary), QualityMetric Hepatitis Quality of Life Questionnaire™ (HQLQ) in the silymarin and antioxidant (SOX) group (P=0.002).

This novel randomised, double-blind, placebo-controlled trial of oral silymarin and oral antioxidants has shown a reduction in hepatic necroinflammation and an improvement in overall mental-health status in a specific CHC population.