

**Sample Proforma for PhD Examination****Sample Thesis Title:**

*“The effect of herbal-derived antioxidants on the contractility properties and function of the heart under oxidative stress studied using an isolated cardiomyocyte model”.*

*(Sample Comments are shown in bold italics)*

**Introduction**

- Was the literature relevant to the topic reviewed in a critical manner?  
***Comment: Yes. More mention could have been made to other data using adult heart cardiomyocytes in addition to neonatal-derived cardiomyocytes.***
- Was previous work presented within an overall conceptual framework and in a systematic way?  
***Comment: Yes. However the recent work of Wang, Das and Smith (2005) on the novel antioxidants derived from some temperate plants, should have been included.***
- Were the hypotheses to be tested and the aims of the research clearly stated?  
***Comment: Mostly. Aims could have been separated better and a numbering system used.***
- Are the nature and extent of the putative original contributions clear?  
***Comment: Mostly.***

**General Comment on the Introduction:**

***The introduction is generally well written and covers the main areas of the research topic. It lacks a few recent references related to the possible mechanism of action of such herbal antioxidants in relation to key indicators of cell functionality. This and other parts of the thesis would benefit from a thorough spelling and syntax (sentence structure) check.***

**ASSESSMENT - Introduction**

<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
<b>EXCELLENT</b>	<b>GOOD</b>	<b>AVERAGE</b>	<b>POOR</b>
Acceptable - With very minor editing	Acceptable - With minor revisions	Acceptable - With major revision	Not acceptable - Major revision and edit – see comments

**Methods**

- Were the methodologies employed detailed adequately?  
***Comment: The examiner acknowledges the difficulty in maintaining, calcium-tolerant, adult cardiomyocytes over extended periods of time for contractility measurements. However, greater care and optimisation of the short-term culture conditions would have greatly extended the number of cells available for experimentation at any one time.***

- Were the methodologies appropriate and their use justified?  
*Comment: The molecular probes to directly report on the generation of free radical in the intracellular environment have been commercially available for some time. Such a strategy would have been far better than much older, less rigorous or specific, TBA (thiobarbituric acid) titrations.*
- Was the instrumentation used appropriately?  
*Comment: Fluorescence measurements using an indicator of intracellular calcium ion fluxes needed to be standardized to provide actual concentrations which could be related to free versus bound forms of calcium ions. Voltages used to induce contractility were excessive and resulted in very high levels of cardiomyocyte mortality, reducing the collection of good data.*
- Were quantitative methods adequately validated? (e.g. accuracy, precision, etc.)  
*Comment: Statistical treatment of some of the data is very weak. Intra- and inter-assay variations should have been better validated. Bonferroni or other multiple comparison tests were not used appropriately in some sections.*

**General Comment on the Methods Section:**

*This section was reasonable well presented, however, the methods used to undertake multiple studies on single cells with respect to increasing doses of the candidate agent, together with the washout protocol, were not described.*

**ASSESSMENT - Methods**

<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
<b>EXCELLENT</b>	<b>GOOD</b>	<b>AVERAGE</b>	<b>POOR</b>
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**Results Chapters**

- Were results presented in adequate detail?  
*Comment: Data representation could have been integrated better by combining many of the similar data sets into composite graphs and figures. Standard figure symbols as used in international journals should have been used. The actual numbers of inter- and intra-sample replicates in certain data sets were confusing.*
- Were limitations inherent in the studies recognised and stated?  
*Comment: To a certain degree the candidate noted that the extent of replication (ie n= values) was on average quite low. This was in part due to the fact acknowledged above that the short-term cell culture conditions had not been adequately optimised.*
- Were variables which might influence the study recognised and either controlled or measured?

**Comment:** *The study would have benefited if the absolute range of the responses of the particular bioassay system employed had been rigorously determined prior to the experimental aspect of testing of herbal extracts.*

- Were the conclusions reached justifiable in the light of the data and the way they were analysed?

**Comment:** *Mostly. A number of the extracts tested gave highly significant results with respect to the magnitude of their individual responses. Thus the conclusions were justified and broadly reflected the thesis aims.*

- Was full use of the collected data made?

**Comment:** *Some data were only discussed in a very peripheral manner. Greater commentary on the similarity of responses with certain extracts sharing similar structures could have been made.*

- Where appropriate, was due credit given to previous workers for ideas developed?

**Comment:** *Yes. Adequate referencing and brief discussion on the work of others were included.*

**General Comment on the Results Section:**

*This section was reasonably well presented, however, the results could have been better presented if a section detailing the optimisation and validation of the experimental set-up with compounds known to have predictable effects on cardiomyocyte contractility (ie positive and negative controls) were presented before the new experimental data.*

**ASSESSMENT - Results**

<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>
<b>EXCELLENT</b>	<b>GOOD</b>	<b>AVERAGE</b>	<b>POOR</b>
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**General Discussion**

- Was new material reported?

**Comment:** *Yes. Some of the extracts tested have not previously been reported as being cardioactive.*

- Would the new material be perceived as a valuable addition to a field of knowledge?

**Comment:** *Further confirmatory experimental validation work would be necessary particularly on the whole, perfused working heart model before its potential value could be ascertained.*

- Do the conclusions overturn or challenge previous beliefs?

**Comment:** *No. They are consistent in that a full survey of cardioactive compounds from numerous sources of bioactives still remains incomplete.*

- Were the findings compared to the known literature and placed in context?

**Comment:** *Yes. The discussion was logical and compared and contrasted the results of the present work with that previously published in the specific area and in the wider context of antioxidant effectors and protectors of cellular function.*

- Does the work open up new ideas for future research?  
*Comment: The bioactives contained in these tested herbal-derived extracts may also have application in other physiological and pathophysiological conditions besides their putative effects on cardiac and cardiovascular function, eg in inflammation and neoplasia.*

### ASSESSMENT - Discussion

A	B	C	D
EXCELLENT	GOOD	AVERAGE	POOR
Acceptable - With very minor editing	Acceptable - With minor revisions	Acceptable - With major revision	Not acceptable - Major revision and edit – see comments

### General Comment on the Thesis (180-200 words)

### FINAL THESIS ASSESSMENT AND GRADING

A	B	C	D
EXCELLENT	GOOD	AVERAGE	POOR
Acceptable - With very minor editing	Acceptable - With minor revisions	Acceptable - With major revision	Not acceptable - Major revision and edit – see comments

#### Summary:

*The broad aims of this thesis were to develop a cellular model for studying reperfusion injury in order to investigate the putative reported protective effects of various antioxidants found in different herbal extracts. Heart cells (cardiomyocytes) were isolated from both adult and neonatal cardiac tissue from the rat and the effects of the test substances were studied by following the contractile properties of such cells while subjecting them to a variety of oxidative stresses thought to mimic reperfusion injury in the heart. The more lipid soluble herbal extracts appeared to induce the greatest protection from injury induced by the stressors used.*

#### Positive points:

*The finding that two of the herbal extracts tested were able to give a synergistic protective effect at very low concentrations, and that this effect occurred rapidly and was of long duration, is an interesting finding with possible future human clinical applications. The breadth of approaches used by the student in this study was particularly impressive.*

#### Negative points:

*Statistical treatment of the data was inappropriate and more significant outcomes from the data were compromised. Adequate controls particularly with regard to the “vehicle” solutions for administration of agents were lacking. There were many spelling, layout and sentence construction errors (particularly placement of the verb within qualifying statements) which made reading and interpretation difficult.*

**Five Questions the candidate could be asked at an interview or a viva session.**

**Question 1.**

*What differences would you expect in the contractility properties to agonists and antagonists for cardiomyocytes isolated from the rat heart in your experimental studies to that of heart cells that may be obtained from biopsied human myocardial tissue during, for example, bypass surgery? How might this impact were such treatments to be contemplated in human studies related to “heart health”?*

**Question 2.**

*In the data for herbal extract #15, you show a dose curve which exhibits a maximum response and then returns to baseline values with increasing doses. Can you please explain this data and the possible effects an excess of this extract (antioxidants) could be having in your experimental system?*

**Question 3.**

*Explain how the differences between chronic in vivo administrations of herbal extracts to that of acute in vitro additions in your studies may have come about in terms of their differential effects on cardiomyocyte contractility?*

**Question 4.**

*The common finding that the more lipid soluble herbal extracts appear to exhibit greater efficacy might imply a role for the cell membrane in facilitating their effects. Could you explain likely mechanisms whereby this could occur?*

**Question 5.**

*Do you believe your results would have any future implications to the possibility of using antioxidants present in herbal-derived extracts in a clinical setting given that proof-of-principal, as opposed to anecdotal evidence is normally required to endorse further clinical trials and testing of such drugs and remedies? (Answer with examples).*