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EXAMINER'S REPORT ON MR. K. THULASI RAMAN'S PHD THESIS

"IMPACT OF EGCG ON MITIGATING HEPATIC STEATOSIS IN HIGH CHOLESTEROL DIET STRESSED YOUNG AND AGED RATS: AN INSIGHT INTO MACROPHAGE INFILTRATION".

I RECOMMEND THAT THE CANDIDATE BE AWARDED THE DEGREE OF DOCTOR OF PHILOSOPHY IN MEDICAL BIOCHEMISTRY WITHOUT FURTHER EXAMINATION.

The candidate has chosen a socially relevant topic for his research study. Hepatic steatosis represents an early reversible stage of disease that is histologically characterized by the accumulation of triglycerides in hepatocytes. In this study the research scholar points out aatherosclerosis is not only a lipid accumulation disorder but also as an inflammatory condition. High fat consumption leads to hepatic steatosis which in turn upregulates atherogenic aspirant genes leading to Atherosclerosis and death. In the present study is aimed to evaluate the impact of hypercholesterolemic stress on liver of young and aged rats and to study the role of Epigallocatechin gallate (EGCG), in combatting hepatic disease in young and aged rat model.

The introduction chapter of this thesis is well written and articulately composed with adequate background information, literature review on the hepatic steatosis, fatty liver, Hypercholesterolemia, cardiovascular complications, and macrophage infiltration, liver inflammatory markers and also various available treatment and therapeutic options and approaches were discussed.

The methodology applied is clear and organized in an appropriate sequence to address the goal of the planned research study to evaluate the potential impact of EGCG on mitigating hepatic steatosis in high cholesterol diet stressed young and aged rats in controlling or treating

cardiovascular disease . The methodology applied is clear and organized in a sequence to address the goal of the planned research study. The candidate has systematically evaluated the hepatic steatosis in high cholesterol diet stressed young and aged rats and the therapeutic effect EGCG was studied through serum lipid and lipoprotein profile, adipocytokines levels, and inflammatory markers TNF α, cell adhesion molecules (CAMs) and NF-kB , NF-AT. The Macrophage infiltration, necrosis and apoptosis were determined in both groups. The examiner would like to point out that the candidate not only focus on the basic biochemical experiments, such as lipid profile, enzyme assay, histopathology etc., but also carried out advanced experiments such as ELISA, protein expression and mRNA expression, which is certainly shows good effort given the chemical and laboratory equipment availability.

This examiner finds this research study represents an exceptional piece of work. This research study is well thought-out and executed with a great extent. The literature review encompasses a wide range of key issues, in an authoritative, critical and well-informed manner. The rationale for the study itself is explained clearly. The investigation is carried out with a high degree of rigorous, in accordance with current standards of good practice for research in this field. Results and discussion were presented well to establish that the early intervention of EGCG during hepatic steatosis and it may safe guard the cardiovascular system by attenuating inflammatory events in the liver. The results obtained are adequate and the data obtained are discussed in the light of relevant available literature. The results are clearly presented in the form of tables, figures and graphs. The statistical analysis has been carried out to present the significance. This research lays a strong basic foundation for the researchers, physicians and industrialists, who may have to further understand, analyze, and take necessary steps to make use, develop and find a newer and much effective treatment for this devastating disease. It is of significance that the candidate was able to achieve this goal in the present work.

The candidate has already made previous publications and presentations from the thesis. However it is a fertile source for many further publications both within and outside India. I would urge the student to move toward publication, in leading peer reviewed journals.

In summary, this study was well planned, efficiently organized, well-written and the style and layout are excellent. The candidate clearly demonstrates creative abilities in evaluating the potential benefit impact of EGCG on mitigating hepatic steatosis in high cholesterol diet stressed young and aged rat model. This study is original and significant contribution to knowledge and

understanding of evaluating the early intervention of EGCG during hepatic steatosis might safe guard the cardiovascular system. This thesis meets the required standard of a doctoral thesis to Justify the award of a Doctor of Philosophy in Medical Biochemistry.

It is without hesitation that I **RECOMMEND** the thesis to be accepted and **MR. K. THULASI RAMAN** may be awarded the degree of Doctor of Philosophy in Medical Biochemistry, University of Madras. I congratulate both the doctoral fellow Mr. K. Thulasi Raman and Dr. P. Kalaiselvi for their hard work and valuable contribution.

A few specific comments may, however, be directed at the candidate during viva interface:

1. On page 31, there are 2 groups of animals, How many animals in each group and are these experiments are repeated? if so, what is the "n" value?

2. On page 32, under materials and methods section, "EGCG treatment starts on 16th day onwards until end of the experimental period". What is the end point and how do you drive the endpoint?

3. Have you done any toxicity study in rats? Does this dose of EGCG affect any other organ? Also, how do you fix the dose 100mg/kg body weigh/day? What is the LD50 value for EGCG and EGCG is pharmaceutical grade?

4. What is the concentration of the primer used for RT PCR and PCR? The annealing temperature of the entire primer list on page 49 is 58-60° C, in that case why the annealing temperature for the PCR is "varies" on page48; you could have used 58 ° C or 60° C as annealing temperature for all the PCR.

External Examiner/Opponent:

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