

**Effect of Celecoxib on Immunological Liver Injury and  
Depression Induced by Specific Liver Antigen and  
Complete Freund's Adjuvant in C57BL/6 Mice**

Thesis for partial fulfillment of the master degree of Pharmacology and  
Therapeutics

**By**

**Esraa Moustafa ELnahas Fotouh Kabil**

(M.B.B.Ch., 2010)

Demonstrator in Pharmacology and Therapeutics Department, Faculty  
of Medicine - Ain Shams University

**Supervised by**

**Prof. Dr. Mahdy Salama Abou Zeid**

Professor of Pharmacology and Therapeutics  
Faculty of Medicine- Ain Shams University

**Prof. Dr. Hala Salah Abdel-Kawy**

Professor of Pharmacology and Therapeutics  
Faculty of Medicine- Ain Shams University

**Dr. Nevien Fekry Abdallah Hendawy**

Lecturer in Pharmacology and Therapeutics  
Faculty of Medicine-Ain Shams University

**Faculty of Medicine  
Ain Shams University  
2016**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالَ

لَسْبَحَانَكَ لَا عِلْمَ لَنَا  
إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ  
الْعَلِيمُ الرَّحِيمُ

صدق الله العظيم

سورة البقرة الآية: ٣٢



## Acknowledgement

*First of all, all gratitude is due to **Allah** almighty for blessing this work, until it has reached its end, as a part of his generous help, throughout my life.*

*Really I can hardly find the words to express my gratitude to **Prof. Dr. Mahdy Salama Abou Zeid** Professor of Pharmacology and Therapeutics, Faculty of Medicine, Ain Shams University, for his supervision, continuous help, encouragement throughout this work and tremendous effort he has done in the meticulous revision of the whole work. It is a great honor to work under his guidance and supervision.*

*I greatly honored to express my sincere appreciation to **Prof. Dr. Hala Salah Abdel-Kawy** Professor of Pharmacology and Therapeutics, Faculty of Medicine, Ain Shams University, for her kind supervision, continuous help and support throughout this work and her tremendous effort in the meticulous revision of the whole work.*

*My words fail to express my sincere appreciation and gratitude to **Dr. Nevien Fekry Abdallah Hendawy** Lecturer of Pharmacology and Therapeutics, Faculty of Medicine, Ain Shams University, for her patience, continuous directions and support throughout this work and her tremendous effort in the meticulous revision of the whole work.*

---

*My sincere appreciation to **Dr. Walaa Baher Mostafa** Lecturer of Histology, Faculty of Medicine, Ain Shams University for providing her time and support to accomplish the histopathological studies.*

*I'm really indepted to **Prof. Dr. Ahmed Abd El-Salam** Professor of Pharmacology and Therapeutics, Faculty of Medicine, Ain Shams University for supplying us with the celecoxib powder as a gift as well known about Prof. Dr. Ahmed Abd El-Salam to help and support the young candidates in need.*

*I am also grateful to my **senior staff, colleagues** in the Department of Pharmacology, Faculty of medicine, Ain Shams University, and to all who offered me any help to fulfill this work,*

*Last but not least, I dedicate this work to **my family**, whom without their sincere emotional support, pushing me forward this work would not have ever been completed.*



**Esraa ELnahas**

---

## **List of Contents**

| <b>Topic</b>                  | <b>Page</b> |
|-------------------------------|-------------|
| List of Tables                | <b>II</b>   |
| List of Figures               | <b>IV</b>   |
| List of Abbreviations         | <b>VIII</b> |
| Introduction& Aim of the work | <b>1</b>    |
| Review of Literature          | <b>9</b>    |
| Materials and Methods         |             |
| *Materials                    | <b>30</b>   |
| *Methods                      | <b>32</b>   |
| Results                       | <b>52</b>   |
| Discussion                    | <b>124</b>  |
| Summary and Conclusion        | <b>143</b>  |
| Abstract                      | <b>148</b>  |
| References                    | <b>151</b>  |
| Arabic summary                | <b>--</b>   |

## List of Tables

| Table | Title                                                                                                                                                                                                                         | Page |
|-------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| 1     | Effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on novelty suppressed feeding test ( <b>A</b> ) latency to feed ( <b>s</b> ) in a model of immunological liver injury and depression in C57BL/6 mice    | 54   |
| 2     | Effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on novelty suppressed feeding test ( <b>B</b> ) home cage feeding ( <b>µg</b> ) in a model of immunological liver injury and depression in C57BL/6 mice | 57   |
| 3     | Effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on tail suspension test in a model of immunological liver injury and depression in C57BL/6 mice                                                         | 60   |
| 4     | Effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on locomotor activity test ( <b>A</b> ) Number of line crossings in a model of immunological liver injury and in C57BL/6 mice                           | 63   |
| 5     | Effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on locomotor activity test ( <b>B</b> ) Number of rearings in a model of immunological liver injury and in C57BL/6 mice                                 | 66   |
| 6     | Effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on forced swimming test: ( <b>A</b> ) latency time (s) in a model of immunological liver injury and depression in C57BL/6 mice                          | 69   |
| 7     | Effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on forced swimming test: ( <b>B</b> ) immobility time (s) in a model of immunological liver injury and depression in C57BL/6 mice                       | 72   |

| <b>List of Tables (continue)</b> |                                                                                                                                                                                                                                      |             |
|----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| <b>Table</b>                     | <b>Title</b>                                                                                                                                                                                                                         | <b>Page</b> |
| <b>8</b>                         | Effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on serum alanine aminotransferase level in the model of immunological liver injury and depression in C57BL/6 mice                                              | <b>76</b>   |
| <b>9</b>                         | Effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on serum aspartate aminotransferase level in the model of immunological liver injury and depression in C57BL/6 mice                                            | <b>79</b>   |
| <b>10</b>                        | Effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on hepatic tissue hydroxyproline level in the model of immunological liver injury and depression in C57BL/6 mice                                               | <b>82</b>   |
| <b>11</b>                        | Histological changes scoring of the liver including Periportal or periseptal interface hepatitis (piecemeal necrosis), Confluent necrosis, Focal necrosis, apoptosis and focal inflammation, Portal inflammation in different groups | <b>100</b>  |
| <b>12</b>                        | Mean±SD of digital image analysis of hepatic fibrosis, caspase-3 positive cells, TNF- $\alpha$ positive cells and NF- $\kappa$ B positive cells distribution in the different groups                                                 | <b>105</b>  |
| <b>13</b>                        | Mean±SD of digital image analysis of hippocampus caspase positive cells and TNF- $\alpha$ positive cells distribution in the different groups                                                                                        | <b>122</b>  |

| <b>List of Figures</b> |                                                                                                                                                                                                                                    |             |
|------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| <b>Figure</b>          | <b>Title</b>                                                                                                                                                                                                                       | <b>Page</b> |
| <b>1</b>               | Schematic Diagram of NF- $\kappa$ B as an Inflammatory Regulator                                                                                                                                                                   | <b>14</b>   |
| <b>2</b>               | Cytokines observed to be induced in the early phases following exposure to CFA                                                                                                                                                     | <b>27</b>   |
| <b>3</b>               | Chemical structure of celecoxib                                                                                                                                                                                                    | <b>30</b>   |
| <b>4</b>               | Picture taken by digital camera demonstrating the exposure of the liver after the mid line laparotomy and flushing portal vein with cold PBS.                                                                                      | <b>35</b>   |
| <b>5</b>               | Diagrammatic illustration of immunization regimen and the behavioral assessment sequence.                                                                                                                                          | <b>37</b>   |
| <b>6</b>               | Novelty-Suppressed Feeding Test                                                                                                                                                                                                    | <b>39</b>   |
| <b>7</b>               | Picture taken by digital camera demonstrating tail suspension test                                                                                                                                                                 | <b>40</b>   |
| <b>8</b>               | Picture taken by digital camera demonstrating locomotor activity                                                                                                                                                                   | <b>41</b>   |
| <b>9</b>               | Picture taken by digital camera demonstrating forced swimming test                                                                                                                                                                 | <b>42</b>   |
| <b>10</b>              | Bar chart illustrating the effect of the celecoxib (7.5mg/kg/d) versus the celecoxib(15mg/kg/d) on novelty suppressed feeding test (A) latency to feed (s) in a model of immunological liver injury and depression in C57BL/6 mice | <b>55</b>   |



## List of Figures (continue)

| Figure    | Title                                                                                                                                                                                                                                                    | Page      |
|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| <b>11</b> | Bar chart illustrating the effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on novelty suppressed feeding test ( <b>B</b> ) home cage feeding ( <b>µg</b> ) in a model of immunological liver injury and depression in C57BL/6 mice | <b>58</b> |
| <b>12</b> | Bar chart illustrating the effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on tail suspension test in the model of immunological liver injury and depression in C57BL/6 mice                                                       | <b>61</b> |
| <b>13</b> | Bar chart illustrating the effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on locomotor activity test: ( <b>A</b> ): no of line crossings in the model of immunological liver injury and in C57BL/6 mice                           | <b>64</b> |
| <b>14</b> | Bar chart illustrating the effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on locomotor activity test: ( <b>B</b> ) no of rearings in the model of immunological liver injury and in C57BL/6 mice                                  | <b>67</b> |
| <b>15</b> | Bar chart illustrating the effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on forced swimming test: ( <b>A</b> ): latency time ( <b>s</b> ) in a model of immunological liver injury and depression in C57BL/6 mice                | <b>70</b> |
| <b>16</b> | Bar chart illustrating the effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on forced swimming test: ( <b>B</b> ) immobility time ( <b>s</b> ) in the model of immunological liver injury and depression in C57BL/6 mice            | <b>73</b> |

## List of Figures (continue)

| Figure              | Title                                                                                                                                                                                                                      | Page           |
|---------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| <b>17</b>           | Bar chart illustrating the effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on serum alanine aminotransferase level (U/L) in the model of immunological liver injury and depression in C57BL/6 mice   | <b>77</b>      |
| <b>18</b>           | Bar chart illustrating the effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on serum aspartate aminotransferase level (U/L) in the model of immunological liver injury and depression in C57BL/6 mice | <b>80</b>      |
| <b>19</b>           | Bar chart illustrating the effect of the celecoxib (7.5mg/kg/d) versus the celecoxib (15mg/kg/d) on hepatic tissue hydroxyproline (mg/g tissue) in the model of immunological liver injury and depression in C57BL/6 mice. | <b>83</b>      |
| <b>20<br/>(A-F)</b> | A photomicrograph of liver sections in C57BL/6 mice stained with H&E X250& X640.                                                                                                                                           | <b>85-87</b>   |
| <b>21<br/>(A-E)</b> | A photomicrograph of liver sections in C57BL/6 mice stained with Masson's trichrome X250.                                                                                                                                  | <b>88-90</b>   |
| <b>22<br/>(A-D)</b> | A photomicrograph of liver sections in C57BL/6 mice stained with Avidin Biotin Peroxidase for Caspase-3, counterstained with Hx X640.                                                                                      | <b>91-92</b>   |
| <b>23<br/>(A-D)</b> | A photomicrograph of liver sections in C57BL/6 mice stained with Avidin Biotin Peroxidase for NF-κB counterstained with Hx X640.                                                                                           | <b>93-94</b>   |
| <b>24<br/>(A-D)</b> | A photomicrograph of liver sections in C57BL/6 mice stained with Avidin Biotin Peroxidase for TNF-α counterstained with Hx X640.                                                                                           | <b>95-96</b>   |
| <b>25<br/>(A-K)</b> | A photomicrograph of hippocampus sections in C57BL/6 mice stained with H&E X125& X640.                                                                                                                                     | <b>106-110</b> |

| <b>List of Figures (continue)</b> |                                                                                                                                                  |                |
|-----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| <b>Figure</b>                     | <b>Title</b>                                                                                                                                     | <b>Page</b>    |
| <b>26<br/>(A-E)</b>               | A photomicrograph of hippocampus sections in C57BL/6 mice stained with Avidin Biotin Peroxidase for Caspase-3, counterstained with Hx X640.      | <b>111-112</b> |
| <b>27<br/>(A-E)</b>               | A photomicrograph of hippocampus sections in C57BL/6 mice stained with Avidin Biotin Peroxidase for NF- $\kappa$ B, counterstained with Hx X640. | <b>113-115</b> |
| <b>28<br/>(A-E)</b>               | A photomicrograph of hippocampus sections in C57BL/6 mice stained with Avidin Biotin Peroxidase for TNF- $\alpha$ counterstained with Hx X640.   | <b>116-117</b> |

## List of Abbreviations

|                                |                                                       |
|--------------------------------|-------------------------------------------------------|
| <b>C57BL/6</b>                 | C57 black 6 mice                                      |
| <b>CLD</b>                     | Chronic liver disease                                 |
| <b>HCV</b>                     | Hepatitis C virus                                     |
| <b>HBV</b>                     | Hepatitis B virus                                     |
| <b>TNF-<math>\alpha</math></b> | Tumor necrosis factor $\alpha$                        |
| <b>NF-<math>\kappa</math>B</b> | Nuclear factor $\kappa$ B                             |
| <b>COX-2</b>                   | Cyclooxygenase-2                                      |
| <b>PGE-2</b>                   | Prostaglandin E2                                      |
| <b>S-100</b>                   | Supernatant of syngeneic liver homogenate at 100.000g |
| <b>CFA</b>                     | Complete freund's adjuvant                            |
| <b>PBS</b>                     | Phosphate buffered saline                             |
| <b>EAH</b>                     | Experimental autoimmune hepatitis                     |
| <b>NSF</b>                     | Novelty-Suppressed Feeding test                       |
| <b>TST</b>                     | Tail suspension test                                  |
| <b>FST</b>                     | Forced swimming test                                  |
| <b>ALT</b>                     | Alanine aminotransferase                              |
| <b>AST</b>                     | Aspartate aminotransferase                            |
| <b>Hyp</b>                     | Hepatic tissue Hydroxyproline                         |
| <b>AIH</b>                     | Autoimmune hepatitis                                  |

## **List of Abbreviations (continue)**

|                        |                                |
|------------------------|--------------------------------|
| <b>LSP</b>             | Liver specific lipoprotein     |
| <b>IFN</b>             | Interferon                     |
| <b>CYP2C9</b>          | Cytochrome P4502C9             |
| <b>eLMA</b>            | Exploratory locomotor activity |
| <b>ANOVA</b>           | One way analysis of variance   |
| <b>LPS</b>             | Lipopolysaccharide             |
| <b>Con A</b>           | Concavalin A                   |
| <b>CCl<sub>4</sub></b> | Carbon tetrachloride           |
| <b>HSCs</b>            | hepatic stellate cells         |

---

*Introduction and  
Aim of the work*

---

## **INTRODUCTION**

Liver is the target of numerous acute and chronic inflammatory processes (**Manns& Obermayer-Straub, 1997**). It is the principal “residence of metabolic clearing” for exogenous drugs and endogenous chemicals that would harm the body such as cholesterol, steroid hormones, fatty acids, and proteins (**Parkinson, 2001**).

Inflammatory liver injury due to viral infection, autoimmune etiology or intoxication with drugs is mediated by the activation of the innate and/or specific immune response. Inflammatory liver injury may also develop as a part of a systemic inflammatory response (**Liu et al., 2006**).

Chronic liver disease (CLD) is usually caused by chronic injury to the liver with different etiologic agents such as hepatotropic viruses including hepatitis C virus (HCV) and hepatitis B virus (HBV), autoimmune hepatitis, alcoholic liver disease, fatty liver, and other etiologic processes (**Weinstein et al., 2011**).

HBV and HCV infections are the most common causes of liver diseases worldwide and both induce immune mediated acute and chronic necroinflammatory liver disease (**Rehermann& Nascimbeni, 2005**). Chronic hepatitis is characterized by the