

***Neurobehavioral study on the possible effect of  
methyl palmitate in rats***

A thesis submitted in partial fulfillment for the requirements of the master  
degree in pharmaceutical sciences

By

***Marwa Mohammad Saeed***

*Bachelor of Pharmaceutical sciences, Ain Shams University, 2000*

*Under the Supervision of:*

***Dr. Azza Abd El-Fattah***

*Professor and Head of Pharmacology and Toxicology Department*

*Faculty of Pharmacy-Girls, Al-Azhar University*

***Dr. Ebtehal EL-Demerdash Zaki***

*Professor and Head of Pharmacology and Toxicology Department*

*Faculty of Pharmacy, Ain Shams University*

***Dr. Hebatalla Ibrahim***

*Lecturer of Pharmacology and Toxicology Department*

*Faculty of Pharmacy-Girls, Al-Azhar University*

***Ain Shams University***

***2013***



# ***Contents***



<b><i>Contents</i></b>	<b><i>page</i></b>
<b><i>List of abbreviations</i></b>	<b><i>VI</i></b>
<b><i>List of Tables</i></b>	<b><i>VIII</i></b>
<b><i>List of Figure</i></b>	<b><i>X</i></b>
<b><u>Abstract</u></b>	<b><i>1</i></b>
<b><u>Introduction</u></b>	<b><i>2</i></b>
<b><i>Depression</i></b>	<b><i>2</i></b>
<b><i>The neurobiology of depression</i></b>	<b><i>4</i></b>
- <b><i>Monoamine hypothesis</i></b>	<b><i>4</i></b>
- <b><i>Diasthesis-Stress hypothesis</i></b>	<b><i>6</i></b>
- <b><i>Two new neuroinflammatory theories of depression</i></b>	<b><i>7</i></b>
<b><i>Treatment of depression</i></b>	<b><i>9</i></b>
<b><i>1- Psychological treatment</i></b>	<b><i>10</i></b>
<b><i>2- Pharmacological treatment</i></b>	<b><i>10</i></b>
<b><i>Animal models of depression</i></b>	<b><i>13</i></b>
<b><i>1- Neurochemical models of depression</i></b>	<b><i>13</i></b>
<b><i>2- Ethological models of depression based on social stress</i></b>	<b><i>14</i></b>
<b><i>3- Ethological models of depression based on</i></b>	
<b><i>enviromental stress</i></b>	<b><i>16</i></b>
<b><i>4- Olfactory bulbectomy</i></b>	<b><i>17</i></b>
<b><i>5- Natural genetic models of depression</i></b>	<b><i>18</i></b>
<b><i>6- Operant response models</i></b>	<b><i>19</i></b>
<b><i>Depression and aggression</i></b>	<b><i>20</i></b>
<b><i>Pharmacological management of aggression</i></b>	<b><i>21</i></b>
<b><i>Methyl Palmitate</i></b>	<b><i>23</i></b>
- <b><i>Pharmacokinetics</i></b>	<b><i>24</i></b>

## ***Preface***

---

- Pharmacodynamics	25
- Toxicity	28
<b><u>Aim of the work</u></b>	29
<b><u>Materials and methods</u></b>	31
1- Materials	31
1.1. Laboratory animals	31
1.2. Drugs	31
1.3. Chemicals and solvents	32
2- Experimental design:	33
2.1. Models of depression	34
2.2. Parameters measured	35
3- Methods	38
3.1. Behavioral assessment	38
3.1.1. Open-field test (OFT)	38
3.1.2. Forced swimming test (FST)	41
3.1.3. Foot shock-induced aggression test (FIA)	44
3.1.4. Predatory aggression test	47
3.2. Biochemical and histological examination	48
3.2.1. Monoamine neurotransmitters analysis	49
3.2.2. Histopathological examination	50
3.2.3. Immunohistochemical assessment	50
4- Statistical analysis	52
<b><u>Results</u></b>	53
Effect of methyl palmitate in open field test	53
Effect of methyl palmitate in forced swimming test	65
Effect of methyl palmitate in footshock-induced aggression test	71

## ***Preface***

---

<i>Effect of methyl palmitate in predatory aggression test</i>	77
<i>Analysis of neurotransmitters using HPLC</i>	80
<i>Histopathological examination of the brain</i>	86
<i>Immunohistochemical examination of iNOS in the brain</i>	98
<i>Immunohistochemical examination of TNF-<math>\alpha</math> in the brain</i>	107
<b><u>Discussion</u></b>	116
<b><u>Summary and conclusion</u></b>	130
<b><u>Recommendations</u></b>	136
<b><u>References</u></b>	137

***List of abbreviations***

<b><i>Alpha 2</i></b>	<b><i>(<math>\alpha_2</math>)</i></b>
<b><i>Central nervous system</i></b>	<b><i>(CNS)</i></b>
<b><i>Cerebrospinal fluid</i></b>	<b><i>(CSF)</i></b>
<b><i>Clonidine</i></b>	<b><i>(CLO)</i></b>
<b><i>Cyclooxygenase</i></b>	<b><i>(COX)</i></b>
<b><i>Dopamine</i></b>	<b><i>(DA)</i></b>
<b><i>Dopaminergic</i></b>	<b><i>(D)</i></b>
<b><i>Fluoxetine</i></b>	<b><i>(FLX)</i></b>
<b><i>Food and Drug Administration</i></b>	<b><i>(FDA)</i></b>
<b><i>Footshock-Induced Aggression Test</i></b>	<b><i>(FIA)</i></b>
<b><i>Forced swimming test</i></b>	<b><i>(FST)</i></b>
<b><i>Gama amino butyric acid</i></b>	<b><i>(GABA)</i></b>
<b><i>Half-life</i></b>	<b><i>(<math>t_{1/2}</math>)</i></b>
<b><i>Hematoxylin and eosin stains</i></b>	<b><i>(H&amp;E)</i></b>
<b><i>Histaminergic</i></b>	<b><i>(H)</i></b>
<b><i>Inducible nitric oxide synthase</i></b>	<b><i>(iNOS)</i></b>
<b><i>Interferon gamma</i></b>	<b><i>(IFN<math>\gamma</math>)</i></b>
<b><i>Interleukin-6</i></b>	<b><i>(IL-6)</i></b>
<b><i>Interleukin-1 beta</i></b>	<b><i>(IL-1<math>\beta</math>)</i></b>
<b><i>Isolation</i></b>	<b><i>(ISO)</i></b>
<b><i>Messenger Ribonucleic Acid</i></b>	<b><i>(mRNA)</i></b>
<b><i>Methyl palmitate</i></b>	<b><i>(MP)</i></b>

## ***Preface***

---

<b><i>Monoamine oxidase</i></b>	<b><i>(MAO)</i></b>
<b><i>Monoamine oxidase inhibitors</i></b>	<b><i>(MAOIs)</i></b>
<b><i>National Institute for Clinical Excellence</i></b>	<b><i>(NICE)</i></b>
<b><i>Nitric oxide</i></b>	<b><i>(NO)</i></b>
<b><i>Nitric oxide synthase</i></b>	<b><i>(NOS)</i></b>
<b><i>Norepinephrine</i></b>	<b><i>(NE)</i></b>
<b><i>Nuclear factor kappa B</i></b>	<b><i>(NF-κB)</i></b>
<b><i>Nucleus accumbens</i></b>	<b><i>(NAc)</i></b>
<b><i>Open-Field Test</i></b>	<b><i>(OFT)</i></b>
<b><i>Phenylethylamine</i></b>	<b><i>(PEA)</i></b>
<b><i>Pro-inflammatory</i></b>	<b><i>(PI)</i></b>
<b><i>Prostaglandin E2</i></b>	<b><i>(PGE2)</i></b>
<b><i>Reversible inhibitors of MAO-A</i></b>	<b><i>(RIMAs)</i></b>
<b><i>Selective serotonin reuptake inhibitors</i></b>	<b><i>(SSRIs)</i></b>
<b><i>Serotonin (5-HydroxyTryptamine)</i></b>	<b><i>(5-HT)</i></b>
<b><i>Tail suspension test</i></b>	<b><i>(TST)</i></b>
<b><i>Transforming growth factor beta</i></b>	<b><i>(TGF-β)</i></b>
<b><i>Tricyclic antidepressants</i></b>	<b><i>(TCA)</i></b>
<b><i>Tumor necrosis factor alpha</i></b>	<b><i>(TNF-α)</i></b>
<b><i>Chemical structure</i></b>	
<b><i>Carbon tetrachloride</i></b>	<b><i>(CCl<sub>4</sub>)</i></b>
<b><i>Chloride</i></b>	<b><i>(Cl<sup>-</sup>)</i></b>

***List of Tables***

<b><i>Table no.</i></b>	<b><i>Title</i></b>	<b><i>Page</i></b>
<b><i>1</i></b>	<b><i>Effect of methyl palmitate treatment on open field test in clonidine and isolation models of depression using male albino rats</i></b>	<b><i>58</i></b>
<b><i>2</i></b>	<b><i>Effect of methyl palmitate on forced swimming test in clonidine and isolation models of depression using male albino rats</i></b>	<b><i>67</i></b>
<b><i>3</i></b>	<b><i>Effect of methyl palmitate on foot shock-induced aggression test in clonidine and isolation models of depression using male albino rats</i></b>	<b><i>73</i></b>
<b><i>4</i></b>	<b><i>Effect of methyl palmitate on predatory aggression test in clonidine and isolation models of depression using male albino rats (rat vs mouse)</i></b>	<b><i>78</i></b>
<b><i>5</i></b>	<b><i>Effect of methyl palmitate on brain neurotransmitters in clonidine and isolation models of depression using male albino rats.</i></b>	<b><i>82</i></b>

---

## ***Preface***

<b>6</b>	<b><i>Effect of methyl palmitate on brain iNOS in the two depression models using male albino rats</i></b>	<b>100</b>
<b>7</b>	<b><i>Effect of methyl palmitate on brain TNF-<math>\alpha</math> in the two depression models using male albino rats</i></b>	<b>109</b>

***List of Figures***

<b><i>Fig. no.</i></b>	<b><i>Title</i></b>	<b><i>Page</i></b>
<b><i>1.</i></b>	<b><i>Open Field Test Apparatus.</i></b>	<b><i>41</i></b>
<b><i>2.</i></b>	<b><i>Forced Swimming Test Apparatus.</i></b>	<b><i>44</i></b>
<b><i>3.</i></b>	<b><i>Footshock-Induced Aggression Test Apparatus</i></b>	<b><i>46</i></b>
<b><i>4.</i></b>	<b><i>Predatory Aggression Test.</i></b>	<b><i>48</i></b>
<b><i>5.</i></b>	<b><i>(5a): Effect of MP treatment on latency time of open field test in clonidine and isolation models of depression.</i></b>	<b><i>59</i></b>
	<b><i>(5b): Effect of MP treatment on ambulation frequency of open field test in clonidine and isolation models of depression.</i></b>	<b><i>60</i></b>
	<b><i>(5c): Effect of MP treatment on rearing frequency of open field test in clonidine and isolation models of depression.</i></b>	<b><i>61</i></b>
	<b><i>(5d): Effect of MP treatment on self-grooming behavior of open field test in clonidine and isolation models of depression.</i></b>	<b><i>62</i></b>
	<b><i>(5e): Effect of MP treatment on urination of open field test in clonidine and isolation models of depression.</i></b>	<b><i>63</i></b>
	<b><i>(5f): Effect of MP treatment on defecation of open field test in clonidine and isolation models of depression.</i></b>	<b><i>64</i></b>

6.	<i>(6a): Effect of MP treatment on climbing score of forced swimming test in clonidine and isolation models of depression.</i>	68
	<i>(6b): Effect of MP treatment on swimming score of forced swimming test in clonidine and isolation models of depression.</i>	69
	<i>(6c): Effect of MP treatment on immobility score of forced swimming test in clonidine and isolation models of depression.</i>	70
7.	<i>(7a): Effect of MP treatment on latency time to fight of foot shock-induced aggression test in clonidine and isolation models of depression</i>	74
	<i>(7b): Effect of MP treatment on jumping frequency of foot shock-induced aggression test in clonidine and isolation models of depression.</i>	75
	<i>(7c): Effect of MP treatment on rearing frequency of foot shock-induced aggression test in clonidine and isolation models of depression.</i>	76
8.	<i>Effect of MP treatment on muricide incidence of predatory aggression test in clonidine and isolation models of depression.</i>	79

9.	<b><i>(9a): Effect of MP treatment on brain NE in clonidine and isolation models of depression.</i></b>	<b>83</b>
	<b><i>(9b): Effect of MP treatment on brain DA in clonidine and isolation models of depression.</i></b>	<b>84</b>
	<b><i>(9c): Effect of MP treatment on brain 5-HT in clonidine and isolation models of depression.</i></b>	<b>85</b>
10.	<b><i>(10a): Normal structure of the meninges (m), cerebral cortex(cc), cerebrum (cr) in control-1 group.</i></b>	<b>88</b>
	<b><i>(10b): Normal structure of cerebellum in control-1 group.</i></b>	
	<b><i>(10c): Normal structure of medulla oblongata in control-1 group.</i></b>	
11.	<b><i>(11a): Focal gliosis (g) and focal haemorrhage (h) of cerebrum in CLO group.</i></b>	<b>89</b>
	<b><i>(11b): Intracellular oedema (o) and demyelination (d) of cerebrum in CLO group.</i></b>	
	<b><i>(11c): Degeneration in the purkenji cells of cerebellum in CLO group.</i></b>	
	<b><i>(11d): Neuronal degeneration of medulla oblongata in CLO group.</i></b>	

12.	<i><u>(12a)</u>: Focal gliosis of cerebrum in CLO+FLX group.</i>	90
	<i><u>(12b)</u>: Degeneration in the purkenji cells of cerebellum in CLO+FLX group.</i>	
	<i><u>(12c)</u>: Neuronal degeneration of medulla oblongata in CLO+FLX group.</i>	
13.	<i><u>(13a)</u>: Focal gliosis of cerebrum in CLO+MP group.</i>	91
	<i><u>(13b)</u>: Normal structure of cerebellum in CLO+MP group.</i>	
	<i><u>(13c)</u>: Normal structure of medulla oblongata in CLO+MP group.</i>	
14.	<i><u>(14a)</u>: Normal structure of meninges, cerebral cortex, cerebrum in MP-1 group.</i>	92
	<i><u>(14b)</u>: Normal structure of cerebellum in MP-1 group.</i>	
	<i><u>(14c)</u>: Normal structure of medulla oblongata in MP-1 group.</i>	
15.	<i><u>(15a)</u>: Normal structure of meninges, cerebral cortex, cerebrum in Control-2 group.</i>	93
	<i><u>(15b)</u>: Normal structure of hippocampus in Control-2 group.</i>	