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# The Possible Effect of Green Tea Extract on Major Depressive Disorder Induced in Mice

**A Thesis**

Submitted to Zoology Department,
Faculty of Science, Alexandria University
in Partial Fulfillment of the Requirements for the
Degree of Master In

**Molecular Biology**

**Presented by**


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**B.Sc. in Molecular Biology, Faculty of Science, Alexandria University, 2016**


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**2022**

# Summary and Conclusion

Major Depressive Disorder (MDD) is the most common psychiatric disorder among adults. MDD is one of the leading causes of disability all over the world. It is a disorder characterized by anhedonia (inability to experience pleasure), cognitive dysfunction, loss of interest, lack of energy, disturbed sleep, prolonged sadness for more than 2 weeks, and high suicidal tendency.

The strong association between stress, mood disorders, and depression has been discussed for over 20 years in which prolonged stress-induced immune dysfunction is known to be a contributing factor to the impact of stress on wellbeing. Neuropsychiatric disturbances are the most defined consequences of prolonged stress exposure, involving depression-like behaviors, and cognitive deficits.

Several hypotheses have been put forward based on the numerous causative factors to explain this multifactorial disorder such as the inflammatory hypothesis. This hypothesis suggests that MDD is mediated by pro-inflammatory cytokines including interleukin-1beta (IL-1β) and links MDD with abnormalities in functional and structural brain anatomy such as the decrease in the hippocampal volumes and disturbance of brain-derived neurotrophic factor (BDNF).

Multiple recommendations accept that moderate to severe depressive episodes should be treated with medication alone or with a combination of medication and psychotherapy. While several new treatment refinements have been introduced over the last decades, their effectiveness has not increased over time.

For years, **Clomipramine** hydrochloride, a tricyclic antidepressant drug known commercially as **Anafranil**, has been effective in treating depressive episodes and decreasing the severity of symptoms. However, Anafranil causes a variety of side effects and takes several weeks to start its action.

The use of herbs or their parts as well as extracts or isolated metabolites has played a vital role in the research of pharmaceuticals, as certain drugs are themselves natural products or their derivatives. In consideration of the complexities of psychological disorders, probably, targeting a single target does not have the same antipsychotic effect as the targeting of several systems. Thus, **herbal medicine** is widely used to treat psychiatric disorders through different mechanisms of action in different systems.

Epigallocatechin gallate (EGCG), the most abundant catechin in green tea, has been known for its antioxidant, neuroprotective, reactive oxygen species suppressor, and cell viability enhancement. Thus, EGCG has been the target of several studies to possess its beneficial effect as a treatment for different diseases and disorders with low side effects.

The present **study aimed** at investigating the antidepressant effect of **epigallocatechin-3-gallate** (EGCG), a natural polyphenolic compound, in mice subjected to chronic unpredictable mild stress (CUMS). To achieve the purpose of the study histological, ultrastructural, immunological, and molecular studies were conducted.

The experimental animals of the study consist of 40 adult male albino Swiss mice (*Mus musculus*) allocated randomly into four groups (10 mice each) and then treated as follows:

Group 1 : Control animals did not receive any treatment.

Group 2 : MDD animal model [subjected to 8 weeks (56 days) of chronic unpredictable mild stress (CUMS)].

Group 3 : MDD animal model-treated with "EGCG" [administered 200 mg /kg b.w. dissolved in 0.2 ml normal saline for two weeks (D63 - D77)].

Group 4 : MDD animal model-treated with "Anafranil" [administered 20 mg/kg b.w., dissolved in 0.2 ml normal saline for two weeks (D63 - D77)].

- In the current study, weight was measured periodically to assess the effect of CUMS on weight gain and appetite.
- After eight consecutive weeks of CUMS, a forced swimming test (FST) was used for assessing depressive symptoms.
- For the immunological study, serum samples were used for quantifying IL-1β protein concentration levels using the enzyme-linked immunosorbent assay (ELISA).
- For the molecular study, the hippocampal CA3 region expression level of BDNF was measured using qRT-PCR analysis technique.

For the histological study, semithin sections of the hippocampus at the area of CA3 zone were fixed in $_4F_1G$ and post-fixed in $OsO_4$ and stained with Toluidine blue.

For transmission electron microscopy study, hippocampus at the area of CA3 zone specimens ($1mm^3$) were fixed in $_4F_1G$ and post-fixed in $OsO_4$, dehydrated in ethanol, infiltrated in propylene oxide, and embedded in Araldite-Epon mixture. Ultrathin sections were picked upon 200 mesh naked copper grids. After being double stained with Uranyl acetate and Lead citrate, the sections were examined under Joel 100 CX Electron Microscope.

## The most important results obtained from the current study:

### 1- Weight measurements observations

In the MDD animal model group, animals showed a significant decrease in weight and loss of appetite after exposure to 8 weeks of CUMS. The MDD mouse model group treated with EGCG for 2 weeks showed an increase in weight but, the increase was insignificant when compared to the MDD animal model group.

### 2- Behavioral observations

In the control group, animals showed a significant decrease in immobility time during the FST test. MDD mouse model group showed a significant increase in immobility time. However, Animals treated with EGCG for 2 weeks showed a significant decrease in immobility time when compared to its corresponding in the untreated animal model group.

### 3- Immunological observations

In the MDD animal model group, IL-1β serum concentration was significantly increased after 8 weeks of CUMS. However, the MDD animal group treated for 2 weeks with EGCG showed a significant decrease in serum IL-1β concentration indicating the anti-inflammatory properties of EGCG.

### 4- Histological observations

**Examination of the CA3 hippocampus sections of the MDD mouse model revealed several abnormalities including:**

a. CA3 zone cell shrinkage

b. Irregular cell membrane

c. Losing cellular structure and morphology

d. Signs of nuclear pyknosis.

**Post-treatment with EGCG showed a significant improvement when compared to the MDD animal model.**

a. Normal morphological features of cells with round nuclei.

b. No signs of cellular damage or pyknosis.

c. An increase in cell number.

**5- Molecular observations**

BDNF expression in the CA3 zone of the hippocampus was significantly decreased due to CUMS. Treatment with EGCG upregulated the expression level of BDNF which showed a significant increase in expression and concentration after 2 weeks of treatment.

**6- Ultrastructural observations**

**The neuropathological hallmarks observed in electron micrographs of the hippocampus of MDD mice include:**

**a.** Signs of pyknosis (apoptosis)

**b.** Cells with clefted nucleus with indistinct cell membrane

**c.** Indistinct nuclear envelope

**d.** An increased number of lysosomes

**e.** Swelling of cytoplasm in some cells

**f.** Myelin sheath deformation

**g.** Synaptic structural abnormalities

**h.** Reduction in the synaptic vesicles number

**Post-treatment with EGCG showed a remarkable improvement when compared to the untreated MDD group.**

**a.** Decreased signs of cellular damage

**b.** Cells with euchromatic nuclei near to the normal state

**c.** Preserved integrity of mitochondria and rER cisternae in CA3 zone cells

**d.** Less noticeable myelin sheath damage

**e.** Increase in the number of synaptic vesicles

## Conclusion and Future Recommendations

**In conclusion,** results obtained in this study emphasize that exposure to 8 weeks of chronic unpredictable mild stress (CUMS) induced behavioral, immunological, histological, and molecular changes in the hippocampus of albino mice. These pathological changes mimic those observed in human Major Depressive Disorder (MDD). These changes include, increased immobility time and behavioral despair, increased concentration of serum interleukin-1beta (IL-1β) indicating the activation of the immune system, and decreased expression of hippocampal brain derived neurotrophic factor (BDNF). At the cellular level a decrease in the size of CA3, cell shape alters, and significant nuclear abnormalities were detected. At the ultra-structure level abnormalities in cell structure, nuclear membrane, mitochondria, lysosomes, myeline sheath, synapse as well as previously mentioned cellular changes indicated apoptosis of many CA3 cells.

Results of the current study indicated that Epigallocatechin gallate (EGCG) is a promising approach for treating MDD and decreasing the severity of its symptoms. Since treatment of MDD mouse model with EGCG for 2 weeks appear promising in decreasing immobility time during forced swimming test (FST), reducing IL-1β serum concentration, regulating BDNF expression in the hippocampus ameliorating behavioral changes, and reducing the cellular damage in CA3 cells. These include nearly normal cell size, normal shape, less nuclear abnormalities in comparison to their corresponding in MDD mice.

The antidepressant-like effect of EGCG is associated with systemic IL-1β inhibition and hippocampal BDNF enhancement, as well as its ability to attenuate neuronal damage caused by CUMS **(Fig. 35)**. Our model may have application for the development of herbal/safe therapy for MDD, however, further studies are recommended to validate EGCG effects on human subjects.

### RECOMMENDATION

It is recommended to administrate daily EGCG, since it showed antioxidant, anti-inflammatory, and neuroprotective effects.

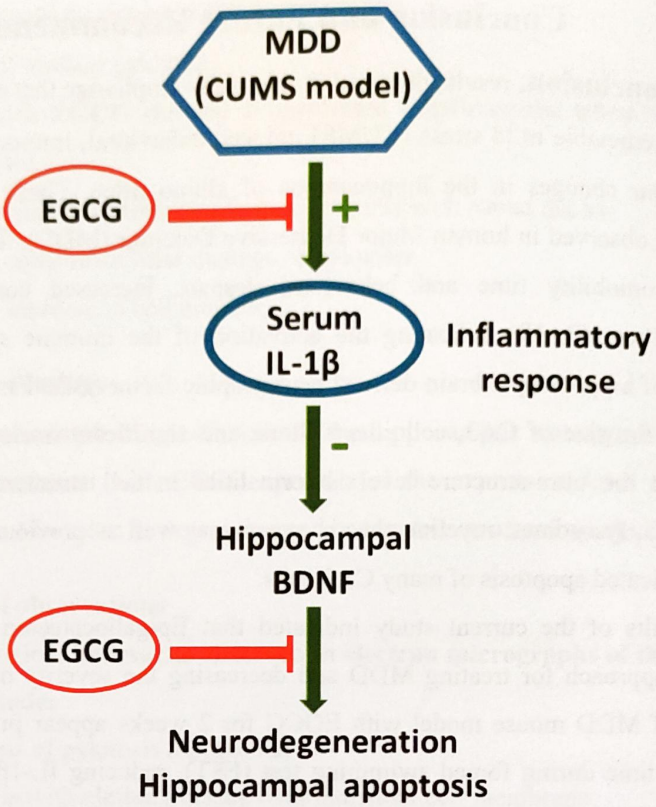


**Fig.37.** Schematic diagram showing the antidepressant actions of EGCG in stressed mice.