

Fetuin-A Ameliorates Lipopolysaccharide-induced Depressive-Like Behavior in Rats Targeting Caspase-1/BDNF/CREB Pathway

Eman H. Basha¹, Heba Faheem¹, Hoda A. Ibrahim², Radwa Ismail³, Monira A. Seleem⁴, Alaa Elkordy⁵, Haidy Khattab^{1*}

1 Medical Physiology Department, Faculty of Medicine, Tanta University, Tanta, Egypt.
2 Medical Biochemistry Department, Faculty of Medicine, Tanta University, Tanta, Egypt
3 Anatomy Department, Faculty of Medicine, Tanta University, Tanta, Egypt
4 Pharmacology Department, Faculty of Medicine, Tanta University, Tanta, Egypt
5 Neuropsychiatry Department, Faculty of Medicine, Tanta University, Tanta, Egypt

Submit Date: 31 Oct. 2022
Revise Date: 26 Nov. 2022
Accept Date: 26 Nov. 2022

Keywords

- Fetuin-A
- BDNF
- Depression
- caspase-1

Abstract

Depression is a mental illness that seriously harms human health. Therefore, it is crucial to create antidepressant treatments that are effective and powerful. Fetuin-A is a multifunctional glycoprotein mainly released by hepatocytes; it has a complex role in inflammatory processes. We aimed to examine the effect of fetuin-A on lipopolysaccharide-induced depressive like behavior in rats. Forty male albino rats were randomly categorized into four groups; **Group I: Control group:** (saline + vehicle) for 10 days, **Group II: Control treated by fetuin-A group:** fetuin-A (100 mg/kg/day) for 10 days, **Group III: Depression group:** 0.5 mg/kg lipopolysaccharide for 10 days, **Group IV: Depression treated with fetuin-A group:** Lipopolysaccharide (0.5 mg/kg) for 10 days followed by fetuin-A (100 mg/kg) for 10 days. Behavioral impairments were evaluated. Brain levels of malondialdehyde (MDA) and glutathione peroxidase (GPX) were estimated. The levels of tumor necrosis factor-alpha (TNF- α), interleukin (IL) IL-6, and brain-derived neurotrophic factor (BDNF), were measured by ELISA. AMPA glutamate receptors (AMPA GluA1&2), caspase-1 and cAMP response element-binding protein (CREB) mRNA expression by real-time PCR were done. Histopathological assessment of hippocampus was done. Our results revealed that fetuin-A effectively ameliorated LPS-induced behavioral tests impairment through increasing BDNF and CREB. Additionally, fetuin-A treatment caused a decrease in the levels of MDA, TNF- α and IL-6 together with concomitant elevation of GPX and upregulation of caspase-1 and AMPAR GluA1&2 expression. We concluded that fetuin-A ameliorates depression-like behaviors of rats by controlling the Caspase-1/BDNF/CREB Pathway signaling pathway, which may serve as a new target for treatment of depression.

INTRODUCTION

Fetuin-A is a multifunctional glycoprotein that is mainly released by hepatocytes. Fetuin-A inhibits the release of proinflammatory mediators induced by bacterial endotoxins in macrophage cultures. It has been demonstrated that fetuin-A acts as a negative acute-phase protein that increases phagocytosis and regulates neutrophil function, all of that support its neuroprotective effect (1). It has a strong affinity, is biologically homogeneous, and has no significant side effects. (2)

Fascinatingly, the anti-inflammatory glycoprotein fetuin-A, also known as alpha2-Heremans-Schmid glycoprotein, prevents the release of proinflammatory cytokines. (3) Fetuin-A is a versatile protein with various physiological functions. (4)

The majority of prenatal organs and tissues, including brain tissue, generally contain fetuin-A protein. Fetuin-A showed neuroprotective effects in animal models of chronic fetal inflammation and cerebral ischemia. The presence of fetuin-A in mature human brain tissue under various physiological and pathological circumstances during development is not well understood yet. Fetuin-A is prevalent in plasma and cerebrospinal fluid and expressed in the liver, brain, kidney, muscle, and bone tissues. (5)

Depression is a mental illness that seriously harms human health and is distinguished by a persistently depressed mood, sluggish thinking, anhedonia, disrupted sleep, and loss of appetite. In severe circumstances, patients may commit suicide and self-harm. (6)

Clinical diagnosis and treatment of depression are challenging due to the multidimensional nature of the disease, which is linked to heredity, environmental, epigenetics, and other factors. The

underlying mechanism of the disease is complex and has not yet been fully understood. Meanwhile, the majority of antidepressant medications have a history of serious side effects and unsatisfactory results. Finding new, potent medications for treating depression that have fewer adverse effects is crucial. (7)

Previous research highlighted that the high level of proinflammatory cytokines released after stress might result in depressive symptoms and that brain inflammations play a significant role in the pathogenesis of depression and anxiety. (8)

One of the most prevalent and well-studied neurotrophins in the human brain is brain-derived neurotrophic factor (BDNF). In addition to being crucial for the healthy growth, development, and plasticity of glutamatergic and GABAergic synapses, BDNF also affects serotonergic and dopaminergic neurotransmission via modulating neuronal differentiation. (9) BDNF levels in the hippocampus are used as biochemical indicators for tracking the onset of depression and its treatment. (10)

In the cerebral cortex and hippocampus, the cAMP response element-binding protein (CREB) is widely distributed. (11) Additionally, CREB is essential for neurogenesis and neuroplasticity. It provides an improved stress response that is important for regaining the structure and function of neurons. (9)

Fang et al. also stated that greater rates of depression had been observed in patients who have infections, and the depressed patients had higher levels of proinflammatory cytokines and lower levels of BDNF, so inflammation has long been thought to be a key role in the development of depression. (12)

Lipopolysaccharide (LPS) is a bacterial endotoxin that is considered one of the toxic bacterial particles that induce brain inflammations leading to depression and anxiety and has been shown to cause depressive-like behaviors in rodents, such as increased immobility in forced swimming tests and tail suspension tests. (13)

Other studies also showed that various pathways linked to depression may be activated by LPS injection. The LPS-induced model is also being used more frequently to test potential novel antidepressants since it causes significant neurochemical changes linked to mood regulation. (14)

Since the potential role of fetuin-A in depression remains unknown, we aim to investigate the influence and the probable mechanisms of fetuin-A administration on depression in rats targeting caspase-1/BDNF / CREB pathway.

1. Material and Methods

2.1. Experimental animals

The study was performed in the animal house of the Faculty of Medicine, Tanta University. The study was carried out on 40 adult male albino rats weighing (180-240 gm) obtained from the experimental animal services house of the Faculty of medicine, Tanta University. The rats were housed in standard, well-ventilated animal cages at room temperature, with free access to water and food throughout the entire period of work. Three rats were the limit allowed per cage in order to prevent overcrowding or lowered cage hygiene. Five times every week, rats were checked for signs of cage aggression or disease. The animal experiments were approved by the Ethical Animal Research Committee of Tanta University, approval number 35963/10/22

2.2. Drugs and chemicals

Fetuin-A & lipopolysaccharide (LPS) were supplied by Sigma–Aldrich Co, (St. Louis, MO).

2.3. Animal groups

After 1 week of acclimatization, the rats were randomly divided into four groups (10 rats each):

Group I (Control group): The rats were treated with intraperitoneal injection of 0.5 ml sodium chloride solution and phosphate-buffered saline daily for 10 days.

Group II (Control treated by fetuin-A group): The rats were treated with 100 mg/kg fetuin-A dissolved in phosphate-buffered saline, which was given daily by intraperitoneal injection for 10 days. (15)

Group III (Depression group): The rats were treated with 0.5 mg/kg lipopolysaccharide, which was dissolved in 0.9% (m/v) sodium chloride solution and was given by intraperitoneal injection for 10 days. (13)

Group IV: Depression treated by fetuin-A group: The rats were treated with 0.5 mg/kg Lipopolysaccharide, which was dissolved in 0.9% (m/v) sodium chloride solution and was given by intraperitoneal injection for 10 days as group III, then 100 mg/kg fetuin-A dissolved in phosphate-buffered saline which was given daily by intraperitoneal injection for another 10 days. (15)

2.4. At the end of the experimental period:

Behavioral tests were done on all groups in the form of forced swimming test and tail suspension test.

2.4.1. Forced swimming test:

Before the test, the rats learned to swim in a training session then separate swimming sessions were required for the rats during the experiment, which was conducted in a cylinder with open Plexiglas, a height of 50 cm, and a diameter of 22.5 cm, which was filled with water to the extent

of 75% of its total volume to prevent the rodents from escaping. Each rat was introduced to the water gradually and carefully. Each rat underwent a 5-minute test period. The floating, swimming, and struggling time in seconds for each rat was recorded. (16)

2.4.2. Tail-suspension test:

Rats were put through a brief period of stress in this test, which is widely used to research depressive-like behavior. Rats were held by the tail at the height of 25 cm off the ground. To keep the rat in a position where they could not climb or flee, adhesive tape was applied to the end of the tail. Within the complete observation period of six minutes, the average mobility duration was noted. Rats were thought to be immobile if they showed full lethargy with no movements. We recorded the immobility time for each rat in seconds. (17)

2.5. Tissue Sampling:

All animals were anesthetized with sodium pentobarbital (50 mg/kg, by intraperitoneal injection) (18) twenty-four hours after the behavioral tests were finished, and then they were all sacrificed by cervical decapitation. The animals' skull vaults were dissected out and temporal lobes were sagittally divided into left and right hemispheres. For histological analysis, the left half of the brains in each group were fixed in 10% neutral buffered formalin for 24 hours. Right hippocampi were carefully extracted and thoroughly washed with 0.9% saline and stored at -80°C and were randomly divided into two divisions. One division was assigned for real-time gene expression. Another division was assigned for biochemical sample analysis after proper homogenization; it was dissected, weighed, homogenized in cold phosphate buffer (pH 7.4), and then centrifuged at 3,000 rpm for 10 min. The

resulting supernatants were separated in clean storage plastic test tubes and stored at -80°C until the time of measurement.

All the remnants of sacrificed animals were discarded in a hygienically and ethical manner by safe disposal measures in the general incinerator of Faculty of Medicine, Tanta University according to the research and safe disposal rules.

2.6. Biochemical analysis:

2.6.1. Redox Status parameters:

Colorimetric determination of malondialdehyde (MDA) levels was determined using the thiobarbituric acid reactive substance assay; the absorbance was read at 532 nm (19), and Enzyme-linked immunosorbent assay (ELISA) was used to detect tissue glutathione peroxidase (Cat #NoMBS265966) as markers of oxidative stress.

2.6.2. Immunoassay of inflammatory markers:

ELISA was used to detect tissue tumor necrosis factor- α (TNF- α) using ELISA kits purchased from MyBiosource (Cat# No MBS355371), and tissue interleukin 6 (IL6) were assayed by ELISA kits purchased from MyBiosource (Cat# NoMBS269892) according to instructions of the manufacturer.

2.6.3. Immunoassay of markers of apoptosis and autophagy:

ELISA was used to detect tissue Bax protein as a marker of apoptosis using ELISA kits which were supplied by (Abcam, USA) (Cat No # ab233624); tissue Beclin-1 as a marker of autophagy was assayed by ELISA kits provided by (Abcam, USA) (Cat No # MOFI00319).

2.6.4. Immunoassay of tissue BDNF:

ELISA was used to detect hippocampal tissue BDNF using ELISA kits provided by (Abcam, USA) (Cat No #ab213899).

2.7. Quantitative assessment of tissue caspase-1, Glut 1& Glut 2 receptors and CREB relative mRNA Expression by quantitative real-time reverse transcription PCR (rt-PCR):

Using a Qiagen RNeasy Total RNA isolation kit (Qiagen, Hiden, Germany), total RNA was extracted from the frozen brain tissue after it had been treated as directed by the manufacturer protocol. The total RNA content, purity, and integrity were calculated using a NanoDrop spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA), and then stored at -80°C , using the OD260 and OD260/280 ratios, respectively. The PCR reactions were carried out using Power SYBR Green PCR Master Mix (Life Technologies, Carlsbad, California, USA).

Caspase-1, CREB, GluA1, and GluA2 mRNA transcript levels were measured in relation to the housekeeping gene GAPDH, which served as a real-time PCR reaction's internal control. Primer 5.0 software was used to create the primer sequences (Table 1). The values of the target and reference genes were used to automatically determine the relative gene expression using the comparative threshold (Ct) approach and the $2^{-\Delta\Delta\text{CT}}$ formula.

2.8. Histopathological assessments:

The left half of each brain were fixed into 10% buffered formalin and processed for paraffin blocks. They were cut into $4\ \mu\text{m}$ thickness-sections using a microtome. Hematoxylin and eosin (H & E) staining was carried out for light microscopic examination and histopathological assessment. (20)

2.9. Statistical Analysis

Mean \pm standard deviation was used to express data. To evaluate the difference between all studied groups, One-way ANOVA was used. To

assess the results between the two groups, post hoc test (Tukey) was used. Results were considered statistically significant with P value <0.05 . SPSS was used to perform statistical tests.

3. Results

3.1. Antidepressant action of fetuin-A improved behavioral tests:

Raising the floating time and reducing swimming and struggling time is considered an indicator of depression. In this research, for the forced swimming test, the results indicated a significantly reduced ($P < 0.05$) duration of struggling and swimming time and a significantly increased time spent floating after LPS administration in the depression group compared with the controls (**Fig.1**). As regard tail suspension test, the immobility time was significantly increased for the depression group compared to the control groups, treatment by fetuin-A significantly decreased immobility time in depression treated with fetuin -A group compared to the depression group (**Fig. 1**).

3.2. Effect of fetuin-A on oxidative stress:

LPS enhances oxidative stress, which is proved by the significant increase in levels of the oxidative stress indicator MDA in the depression group in comparison with controls ($P < 0.05$). Furthermore, levels of GPx, a principal intercellular antioxidant, were reduced drastically in depression group in comparison with controls ($P < 0.05$). In the depression treated with fetuin-A group, MDA level significantly fell and GPx, level rose in comparison with depression group ($P < 0.05$) (**table 2**).

3.3. Effect of fetuin-A on proinflammatory cytokines & apoptotic and autophagy biomarkers:

The tissue levels of proinflammatory cytokines IL-6 and TNF- α were higher in the depression group than in control rats ($P < 0.05$). Depression treated with fetuin-A group showed a significant decrease in IL-6 and TNF- α levels compared with depressed rats ($P < 0.05$). (table 2)

Regarding apoptotic biomarker administration of LPS induced a significant increase in tissue Bax level compared to the control group. On the other hand, fetuin-A caused a significant decrease in

tissue Bax level compared to the depression group ($P < 0.05$) (table 3).

Beclin-1, an autophagy marker, showed decreased levels in the depression group compared to the control group. In contrast, treatment of fetuin-A induced a significant elevation ($P < 0.05$) in Beclin-1 level compared with depressed rats (table 3).

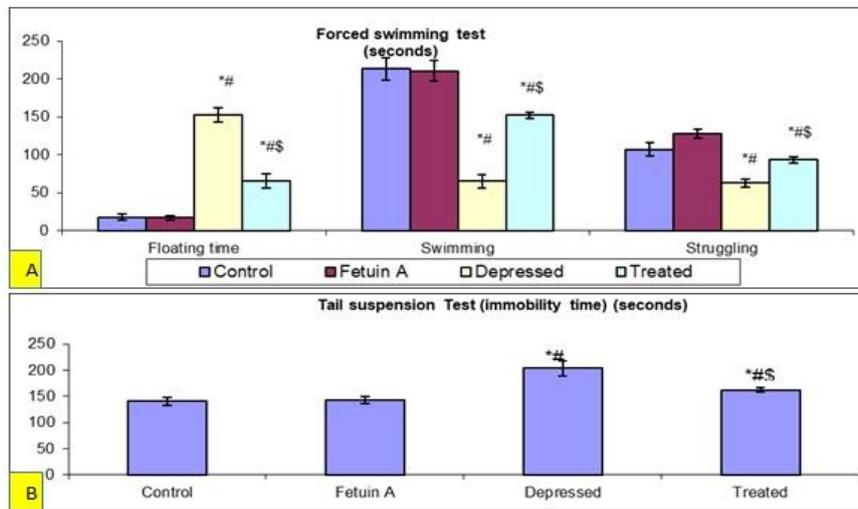


Figure 1: Graphical presentation of the effect of fetuin-A on behavioral test

A) Forced swimming test in the studied groups (floating time, swimming time, struggling time)

B) Tail suspension test in the studied groups (immobility time)

Group I(control), group II(fetuin-A), group III(depressed), group IV(treated)

* denote a statistically significant difference at ($P < 0.05$) compared to group I

denote a statistically significant difference at ($P < 0.05$) compared to group II

\$ denote a statistically significant difference at ($P < 0.05$) compared to group III

Table 1: Real-time PCR (qPCR) primer pairs

Gene	Nucleotide accession No.	Primers	Amplicon size
<i>Caspase -1</i> (ID: 1675178545)	NM_001223.5	Forward: 5'- TCCGTTATTCCGAAAGGGGC-3' Reverse: 5'- ATAGCTGGGTTGTCCTGCAC-3'	128
<i>CREB</i> (ID: 1519313108)	NM_004379.5	Forward: 5'- GCACTATTGCCCTGGAGTT-3' Reverse:: 5'- ACCTCTCTCTTCGTGCTGC-3'	85
GluA1 (ID: 1890265950)	NM_001114183.2	Forward: 5'- TCCCAACAATATCCAGATAGGG-3' Reverse:: 5'- AAGCCGCATGTTCTGTGATT-3'	63
GluA2 (ID: 1825761143)	NM_000826.6	Forward: 5'- AATGGACGTGTTATGACTCCAGA-3' Reverse:: 5'- CTGACATTCATTCCCATGCCA-3'	83
GAPDH (ID: 2295558031)	NM_008084.4	Forward: 5'- TTCACCACCATGGAGAAGGC -3' Reverse:: 5'- TGATGGCATGGACTGTGGTC -3	241

Table (2): Effect of fetuin-A on oxidative stress & pro-inflammatory cytokines

	Group I	Group II	Group III	Group IV	F. test	p. value
MDA nmol/gm tissue	2.82 ± 0.53	2.81 ± 0.44	8.51 ± 0.66 ^{*#}	4.21 ± 0.63 ^{*#S}	221.317	0001 [*]
GPX μmol /gm tissue	24.41 ± 1.80	23.75 ± 0.82	13.07 ± 1.05 ^{*#}	19.84 ± 1.14 ^{*#S}	171.677	0001 [*]
TNF alpha pg/mg tissue	14.77 ± 1.31	13.85 ± 1.78	39.29 ± 4.37 ^{*#}	23.49 ± 2.77 ^{*#S}	175.688	0001 [*]
IL-6 ng/gm tissue	5.79 ± 0.81	6.04 ± 0.66	25.90 ± 3.53 ^{*#}	8.38 ± 0.54 ^{*#S}	270.168	0001 [*]

Data are represented as mean ± SD

* denote a statistically significant difference at (P < 0.05) compared to group I

denote a statistically significant difference at (P < 0.05) compared to group II

S denote a statistically significant difference at (P < 0.05) compared to group III

Table (3): Effect of fetuin-A on apoptotic and autophagy biomarkers:

	Group I	Group II	Group III	Group IV	F. test	p. value
Beclin 1 ng/g tissue	8.77±0.47	8.78 ± 0.44	2.82 ± 0.35 ^{*#}	5.89 ± 0.53 ^{*#S}	397.381	0001 [*]
BAX ng/g tissue	2.79±0.44	2.85 ± 0.39	9.07 ± 0.37 ^{*#}	4.82 ± 0.27 ^{*#S}	615.995	0001 [*]

Data are represented as mean ± SD

* denote a statistically significant difference at (P < 0.05) compared to group I

denote a statistically significant difference at (P < 0.05) compared to group II

S denote a statistically significant difference at (P < 0.05) compared to group III

3.4. Fetuin-A treatment ameliorates LPS-induced increase of caspase-1 expression and attenuation of BDNF-CREB pathway in the hippocampus

Rats in the depression group showed increased caspase-1 levels and downregulation of CREB expression levels in the hippocampus compared to the control group. Treatment with fetuin-A significantly decreases caspase-1 in the hippocampus (table 4). Moreover, Fetuin-A administration increased CREB expression in the hippocampus (Fig.2).

Furthermore, Rats in the depression group showed downregulation of BDNF, fetuin-A administration increased BDNF expression in the hippocampus (table 4).

3.5. Fetuin-A enhances the stability of AMPA glutamate receptors AMPARs GluA1&2 in the hippocampus

We investigate AMPAR expression in the hippocampal region. As shown in (Fig. 2), as compared to the control group, LPS treatment significantly reduced the levels of GluA1 and GluA2 mRNA in the hippocampus. The LPS-induced decrease of GluA1 and GluA2 in the hippocampal region was greatly mitigated by fetuin-A treatment. It is widely known that the AMPARs' phosphorylation controls synaptic effectiveness. These findings suggest that fetuin-A increases the expression of AMPARs in the hippocampal postsynaptic membrane.

3.6. Histopathological results

The CA1 in the control group and control treated by fetuin-A group (groups I & II) revealed small pyramidal cells with rounded vesicular nuclei, prominent nucleoli and apical dendrites. The CA1 region in the depression group (groups III) rats exhibited small shrunken pyramidal cells with an acidophilic cytoplasm and pyknotic nuclei and the apical dendrites of the cells were lost. CA1 in the

depression treated with fetuin-A group (groups IV) retained an apparently normal appearance showing the small pyramidal cells with rounded - vesicular nuclei and apical dendrites. Also, a few small shrunken pyramidal cells with pyknotic nuclei were detected (**Fig. 3**).

In the control group and control treated by fetuin-A group (groups I & II), The CA3 hippocampal pyramidal cells were well arranged in 3-5 compact layers with rounded vesicular nuclei. In the Depression group (groups III), The thickness of CA3 reduced with distortion of compact layers. Shrunken pyramidal cells with deeply stained nuclei and pericellular halo & neuropil vacuolations were detected. These abnormalities were relatively reduced in CA3 in the depression

treated with fetuin-A group (groups IV) exhibiting large pyramidal cells with rounded vesicular nuclei and acidophilic cytoplasm. Also, a few shrunken pyramidal cells with pyknotic nuclei were displayed (**Fig. 4**).

The dentate gyrus (DG) in the control group and control treated by fetuin-A group (groups I & II) contained numerous densely packed granular cells with rounded vesicular nuclei. In sections of the depression group (groups III), the dg exhibited numerous degenerated cells with pyknotic nuclei. in depression treated with fetuin-A group (groups IV), the DG exhibited multiple mature granular cells and few degenerated cells. An apparently little neuropil vacuolation was demonstrated (**Fig. 5**).

Table (4): Effect of fetuin-A on caspase-1 & BDNF levels:

	Group I	Group II	Group III	Group IV	F. test	p. value
Caspase -1 mRNA expression	0.90±0.05	0.92 ± 0.05	1.50 ± 0.17 ^{*#}	1.06 ± 0.13 ^{*#S}	63.466	0001 [*]
BDNF level (pg/mg protein)	9.03±0.49	9.13 ± 0.47	5.41 ± 0.25 ^{*#}	7.52 ± 0.24 ^{*#S}	207.240	0001 [*]

Data are represented as mean ± SD

* denote a statistically significant difference at (P < 0.05) compared to group I

denote a statistically significant difference at (P < 0.05) compared to group II

\$ denote a statistically significant difference at (P < 0.05) compared to group III

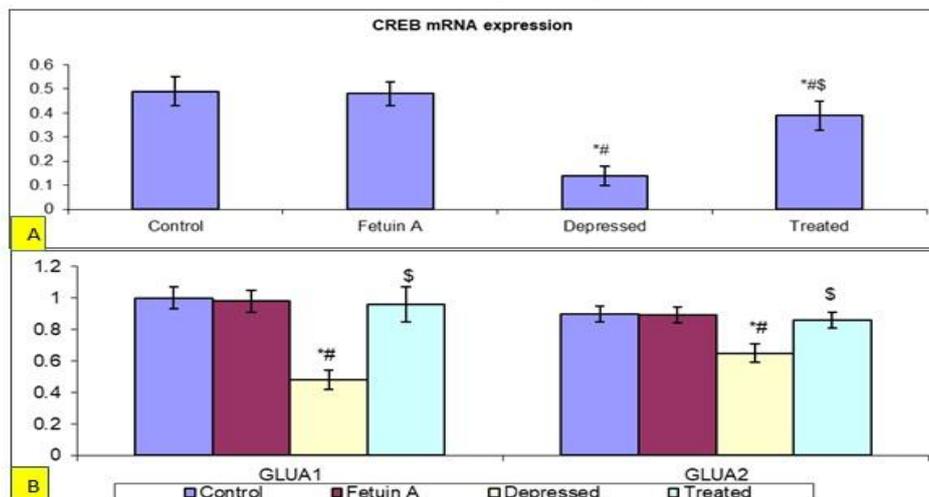


Figure 2: Graphical presentation of the Effect of Fetuin-A on

A) CREB mRNA expression in in the hippocampus

B) AMPARs Glut 1&2 mRNA expression in the hippocampus

Group I(control), group II(fetuin-A), group III(depressed), group IV(treated)

Data are represented as mean ± SD

* denote a statistically significant difference at (P < 0.05) compared to group I

denote a statistically significant difference at (P < 0.05) compared to group II

\$ denote a statistically significant difference at (P < 0.05) compared to group III

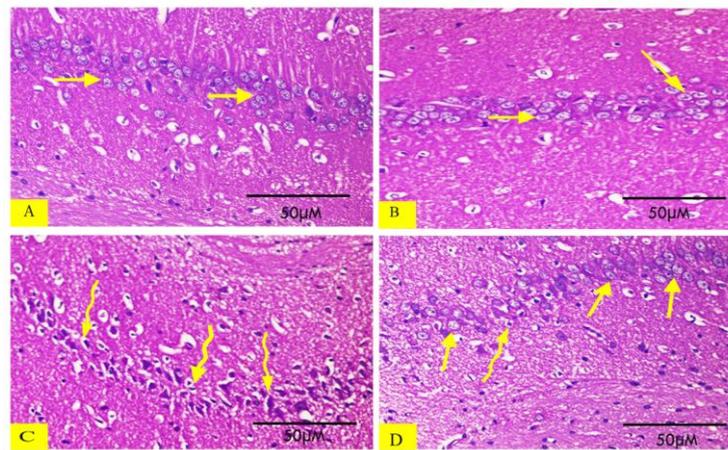


Figure 3: A photomicrograph of the hippocampus CA1 of the different studied groups stained with H&E staining.

CA1 area in group I & II (A &B) showing pyramidal cells having rounded vesicular nuclei with prominent nucleoli and apical dendrites (arrows). CA1 in group III (C) showing multiple small shrunken pyramidal cells with pyknotic nuclei and acidophilic cytoplasm. The apical dendrites of the cells were lost. (wavy arrows). CA1 in group IV (D) showing small pyramidal cells with rounded vesicular nuclei and acidophilic cytoplasm (arrows) and a few small shrunken pyramidal cells with pyknotic nuclei (wavy arrow) (X 400, scale bar 50 µm).

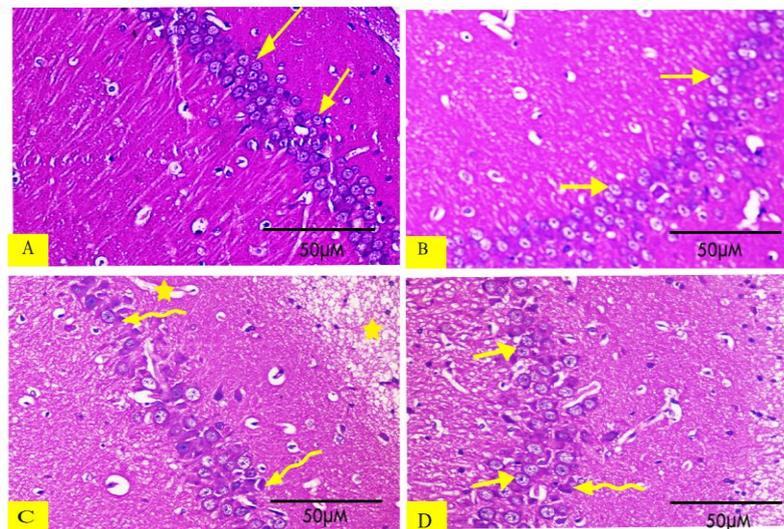


Figure 4: A photomicrograph of the hippocampus CA3 of the different studied groups stained with H&E staining.

CA3 area in group I & II (A &B) contains 3-5 compact layers of pyramidal cells with rounded vesicular nuclei and prominent nucleoli (arrows). CA3 in group III (C) showing reduction in thickness and distortion of compact layers. Shrunken pyramidal cells with deeply stained nuclei and pericellular halo (wavy arrows) & neuropil vacuolations (stars) were detected. CA3 in group IV (D) showing large pyramidal cells with rounded vesicular nuclei and acidophilic cytoplasm (arrows). Also, a few shrunken pyramidal cells with pyknotic nuclei were seen (wavy arrow) (X 400, scale bar 50 µm).

4. Discussion

Research on the pathogenesis and therapy of depression has been a focus of interest. It is crucial to create antidepressant treatments that are effective and powerful. (21) Previous research has shown that long-term stress exposure can change gene expression and cause functional alterations

in the brain circuit, resulting in abnormal behavior (22).

The hippocampus is a key brain region in the neuronal circuit that regulates cognitive and emotional behaviors. Chronic stress leads to decrease of the expression of AMPAR and miniature the amplitude of the hippocampus's excitatory postsynaptic current. (10)

In this study, we demonstrated that fetuin-A exerted an antidepressant effect on LPS-induced depression rat models. Our results indicate that the antidepressant-like effects of fetuin-A may depend on anti-inflammatory activity, antioxidative, antiapoptotic and stimulation of autophagy. Furthermore, we hypothesized that fetuin-A may suppress inflammation brought on by caspase-1 in the hippocampus, hence enhancing the BDNF-CREB pathway. As a consequence, GluA1's Ser845 was phosphorylated more frequently, improving the stability of surface AMPARs in the hippocampus and ultimately controlling depressive-like behaviors in rats.

There is growing evidence that suggests that inflammation may contribute to the etiology of depression. (23) Synaptic plasticity is impacted by proinflammatory cytokines, which is linked to anhedonia, behavioral despair, and cognitive decline. (24) LPS causes depressive-like behaviors by blocking BDNF-dependent synaptic plasticity and increasing the production of proinflammatory cytokines in activated microglia. (25)

In addition to other physiological functions, BDNF is essential for neurogenesis and synaptic plasticity. BDNF levels are noticeably reduced in depressed individuals compared to healthy controls, although they can be significantly increased by antidepressant therapy. (26) Depressed animals have lower levels of BDNF, which can be restored by antidepressants (27). Additionally, elevated proinflammatory cytokines lower levels of BDNF and decreased neuronal plasticity that depends on BDNF. (10)

According to previous studies, depression alters BDNF-related signaling pathways. CREB, one of the most commonly studied transcription factors

involved in the genesis of depressive and antidepressive reactions, controls BDNF expression through various signaling pathways. In addition to controlling the nucleus, CREB is a crucial regulator of other intracellular signaling pathways in the nervous system. (28)

Psychosis and cognitive behavior impairment are associated with CREB signal transduction in the hippocampus. Additionally, CREB's primary downstream target gene is BDNF, and CREB is the most common neurotrophin in brain dysfunction. Additionally, BDNF is regarded as one of the target molecules that has received the most attention for regulating the survival, differentiation, and brain plasticity of the peripheral and central nervous systems. (29)

Our study showed that the expression levels of BDNF and CREB in the hippocampus declined in rat models of LPS-induced depression. However, the CREB/BDNF signaling pathway was regulated, the cell apoptosis was weakened, and the depression-like behaviors were improved after the treatment with fetuin-A, indicating that fetuin-A probably exerts an antidepressant-like effect by modulating the CREB/BDNF signaling pathway.

This is in agreement with Liao et al., who stated that in the hippocampus of the depression model, the expressions of the BDNF and CREB/BDNF signaling pathways, including BDNF, CREB, phosphorylated ERK1/2, phosphorylated AKT, and TrkB (a BDNF receptor), substantially decrease. (30)

AMPARs are glutamate receptors composed of a combination of four subunits, GluA1–4, that contribute specific properties related to receptor function, including kinetics, permeability, and trafficking. (31) The pathophysiology of depression involves glutamatergic

neurotransmission, which is controlled by the BDNF signaling pathway. (32) It was found that BDNF increased AMPAR transcription and protein expression. (33) The delivery of AMPARs was linked to the availability of BDNF, according to further evidence gathered utilizing various biochemical and physiological approaches. (33) We thus examined the expression of AMPARs in the hippocampus. Our result revealed a significant reduction in the surface expression of AMPARs in the hippocampus of depressed rats, and these changes were reversed by fetuin-A therapy which reversed the LPS-induced reduction in hippocampus GluA1 and GluA2 mRNA. These findings imply that AMPARs function may help explain fetuin-A's antidepressant-like effects. Zhang et al. demonstrated that the antidepressant medication tianeptine works by stimulating the BDNF-TrkB pathway and restoring defective AMPAR mobilization. (34)

Neurodegenerative and depressive diseases are frequently caused by etiologic variables such as neural inflammation and apoptosis. (35). Zhao et al. concluded that Proinflammatory cytokines (TNF-, IL-1, and IL-6) and COX-2 were overproduced in the hippocampus tissue after exposure to LPS. (36) Increased production of proinflammatory cytokines has been linked to the inflammatory reaction that occurs after exposure to LPS. (37) The administration of fetuin-A, however, showed neuroprotective properties by inhibiting the inflammatory cascade.

Fetuin-A has been shown in numerous studies to be an effective anti-acute phase protein in sepsis; it has a much more complicated role in inflammatory processes and it may be a protein that stimulates or suppresses the acute phase depending on the specific triggers. TNF-alpha,

interleukin (IL)-1, IL-6, and IFN- γ are examples of proinflammatory cytokines implicated in the early stage of inflammation. (38) Our study revealed that LPS increased the level of inflammatory cytokines TNF-alpha, IL6 but treatment with fetuin-A decrease these cytokines indicating the anti-inflammatory effect of fetuin-A, this in agreement with Kim et al. who stated that LPS causes the immune system to become activated by the release of cytokines such as IL-6 and TNF- α (39). TNF- α and IL-1 β levels are reduced by fetuin-A during experimentally induced intestinal ischemia/reperfusion. (1)

Additionally, oxidative stress is crucial to the pathophysiology and development of psychiatric disorders like bipolar disorder and depression. (40) One of the causes of depression is frequent or unpredictable stress, which increases the production and release of ROS, depletes the body's supply of natural antioxidant proteins, and causes neurodegeneration and necrosis. (41) The current work investigated LPS-induced hippocampus oxidative damage and found that MDA levels were elevated and the antioxidant system was suppressed as evidenced by decreased antioxidant molecule activities, including GPx, fetuin-A administration protected hippocampal tissues by increasing endogenous antioxidants and preventing the production of pro-oxidants. These results align with those of Asadi et al. (42) who discovered that fetuin-A increased total antioxidant capacity levels and also inhibited the production of MDA in ovarian tissue. Furthermore, according to El-Malkey et al., fetuin-A downregulates tissue MDA in experimentally induced intestinal ischemia/reperfusion. (1)

Moreover, in this study, we found that LPS induce neuronal apoptosis, evidenced by an increase in caspase-1 expression in brain tissue and also an increased level of Bax. However, fetuin-A treatment significantly downregulates the level of Bax and Caspase-1, preventing neuronal apoptosis.

In hyperlipidemic circumstances, circulating fetuin A plays crucial roles in cell dysfunction and apoptosis. (43) Fetuin-A may act as a protective agent against tissue damage through the prevention of apoptosis; it may indirectly lessen hepatic apoptosis by inhibiting TNF- α , which could induce hepatic apoptosis. (2)

Previous studies have demonstrated that oxidative stress and autophagy pathways interact in various diseases. (44) There is a connection between autophagy and several diseases, including stress-related diseases like depression. (45) Fetuin-A induce autophagy through upregulation of the level of beclin 1 in the hippocampus, thus improving the depression-like behavior of depressed rats. Soares et al. (46) stated that an effective autophagy mechanism is necessary for cells to survive stress; Evidence suggests that cell death and abnormal function may also be caused by inadequate autophagosome processing. Fetuin-A protects against experimentally induced intestinal ischemia/reperfusion through suppression of autophagic cell death. (1)

Other studies revealed that LPS administration causes depressive-like behaviors in rodent models, including increased immobility during the FST and TST, a reduction in saccharin solution consumption, the inhibition of sexual behavior, and a reduction in cocaine-induced location preference. (44) We used the FST and TST in our work, which have been widely used for the

preclinical screening of antidepressants, to assess depressive-like behaviors in rats. Here, we found that rats given LPS showed an increase in floating duration, decreased swimming and struggling time in the FST, and an increase in immobility time in the TST. Additionally, we showed that fetuin-A reduces depressive-like behaviors in the rat model of depression caused by LPS.

Our histopathology findings demonstrated that fetuin-A alleviated the Lipopolysaccharide-induced neurodegeneration in hippocampal regions. Rubah et al. demonstrated that LPS-induced depression model micrographs showed the production of vacuoles, swollen cytoplasm, and scalloped morphology of neurons with cytoplasmic eosinophilia and nuclear basophilia (47). Another study confirmed that LPS reduced the expression of survival neurons (48). Fetuin-A functions as a neuroprotective and anti-inflammatory molecule in the brain, as well as having pleiotropic effects on target organs (49).

5. Conclusion

Fetuin-A ameliorates depression-like behaviors of rats by controlling the CREB/BDNF signaling pathway, which may serve as a new target for the treatment of depression. These findings provide new insights into fetuin A for the treatment of depression.

6. Recommendation

The results provide a novel insight into the potential use of fetuin-A to expand a therapeutic and protective window against depression-related effects, an area that still requires further study, which still needs more research. Additionally, fetuin-A analogues could be developed and used as potential therapeutic targets for neurological impairments.

5. Declarations and statements

5.1. Ethics approval and consent to participate:

We conducted the study protocol according to The Local Committee of Research and Medical Ethics of the Faculty of Medicine, Tanta University.

5.2. Consent to publication: Not Applicable.

5.3. Availability of data and material: The corresponding author can provide the datasets used and/or analyzed during the current work upon request.

5.4. Competing interests: The authors declare to have no conflicts of interest.

6. Funding: The authors received no specific funding for this work.

7. Authors' contributions: All authors contributed to the data analysis and interpretation of the data, drafted, and revised the manuscript, and approved the final version of the manuscript.

8. Acknowledgment

We want to acknowledge our colleagues, and laboratory workers who helped us in this work

9. References

- 1-El-Malkey NF, Alsemeh AE, Ashour WM, Hassan NH, Edrees HM. Fetuin-A exerts a protective effect against experimentally induced intestinal ischemia/reperfusion by suppressing autophagic cell death. *Exp Biol Med (Maywood)*. 2021;246(11):1307-1317. doi:10.1177/1535370221995207
- 2- Zhang P, Shen H, Huang J, et al. Intraperitoneal administration of fetuin-A attenuates D-galactosamine/lipopolysaccharide-induced liver failure in mouse. *Dig Dis Sci*. 2014;59(8):1789-1797. doi:10.1007/s10620-014-3071-0
- 3- Barış Ö, Gacar G, Halbutoğulları SZ, Fındık O, Korun ZE, Yazır Y, Utkan T, Çelikyurt Fİ, Benli ED. The role of fetuin-A in vascular aging in the rat aorta. *Turkish Journal of Vascular Surgery*. 2022;31(1).
- 4-Ochieng J, Nangami G, Sakwe A, et al. Impact of Fetuin-A (AHSG) on Tumor Progression and Type 2 Diabetes. *Int J Mol Sci*. 2018;19(8):2211. Published 2018 Jul 29. doi:10.3390/ijms19082211
- 5- Heinen MC, Babler A, Weis J, et al. Fetuin-A protein distribution in mature inflamed and ischemic brain tissue. *PLoS One*. 2018;13(11):e0206597. Published 2018 Nov 9. doi:10.1371/journal.pone.0206597
- 6- Howard DM, Adams MJ, Shirali M, et al. Genome-wide association study of depression phenotypes in UK Biobank identifies variants in excitatory synaptic pathways [published correction appears in *Nat Commun*. 2021 Mar 25;12(1):2012]. *Nat Commun*. 2018;9(1):1470. Published 2018 Apr 16. doi:10.1038/s41467-018-03819-3.
- 7- Chen Y, Yang X, Li H, Fang J. Red Raspberry Extract Decreases Depression-Like Behavior in Rats by Modulating Neuroinflammation and Oxidative Stress. *Biomed Res Int*. 2022; 2022:9943598. Published 2022 Jul 2. doi:10.1155/2022/9943598
- 8- Arab Z, Hosseini M, Mashayekhi F, Anaeigoudari A, Zataria A. Zataria multiflora extract reverses lipopolysaccharide-induced anxiety and

- depression behaviors in rats. *Avicenna J Phytomed.* 2020;10(1):78-88.
- 9- Kandezi N, Mohammadi M, Ghaffari M, Gholami M, Motaghinejad M, Safari S.** Novel Insight to Neuroprotective Potential of Curcumin: A Mechanistic Review of Possible Involvement of Mitochondrial Biogenesis and PI3/Akt/ GSK3 or PI3/Akt/CREB/BDNF Signaling Pathways. *Int J Mol Cell Med.* 2020;9(1):1-32. doi:10.22088/IJMCM.BUMS.9.1.1
- 10- Li MX, Zheng HL, Luo Y, et al.** Gene deficiency and pharmacological inhibition of caspase-1 confers resilience to chronic social defeat stress via regulating the stability of surface AMPARs. *Mol Psychiatry.* 2018;23(3):556-568. doi:10.1038/mp.2017.76
- 11- Gu X, Liu L, Shen Q, Xing D.** Photoactivation of ERK/CREB/VMAT2 pathway attenuates MPP⁺-induced neuronal injury in a cellular model of Parkinson's disease. *Cell Signal.* 2017;37:103-114. doi:10.1016/j.cellsig.2017.06.007
- 12-Fang K, Li HR, Chen XX, et al.** Quercetin Alleviates LPS-Induced Depression-Like Behavior in Rats via Regulating BDNF-Related Imbalance of Copine 6 and TREM1/2 in the Hippocampus and PFC [published correction appears in *Front Pharmacol.* 2020 Apr 15;11:518]. *Front Pharmacol.* 2020;10:1544. Published 2020 Jan 17. doi:10.3389/fphar.2019.01544
- 13- Chen J, Zhou T, Guo AM, et al.** Metformin Ameliorates Lipopolysaccharide-Induced Depressive-Like Behaviors and Abnormal Glutamatergic Transmission. *Biology* (Basel). 2020;9(11):359. Published 2020 Oct 26. doi:10.3390/biology9110359
- 14- Cordeiro RC, Chaves Filho AJM, Gomes NS, et al.** Leptin Prevents Lipopolysaccharide-Induced Depressive-Like Behaviors in Mice: Involvement of Dopamine Receptors. *Front Psychiatry.* 2019;10:125. Published 2019 Mar 12. doi:10.3389/fpsyt.2019.00125
- 15- Li W, Zhu S, Li J, et al.** A hepatic protein, fetuin-A, occupies a protective role in lethal systemic inflammation. *PLoS One.* 2011;6(2):e16945. Published 2011 Feb 8. doi:10.1371/journal.pone.0016945
- 16- Fahmy HM, Mohamed ER, Hussein AA, Khadrawy YA, Ahmed NA.** Evaluation of the therapeutic effect of mesoporous silica nanoparticles loaded with Gallic acid on reserpine-induced depression in Wistar rats. *BMC Pharmacol Toxicol.* 2022;23(1):40. Published 2022 Jun 15. doi:10.1186/s40360-022-00579-1
- 17- Pignataro P, Dicarolo M, Zerlotin R, et al.** Antidepressant Effect of Intermittent Long-Term Systemic Administration of Irisin in Mice. *Int J Mol Sci.* 2022;23(14):7596. Published 2022 Jul 8. doi:10.3390/ijms23147596
- 18- Allen-Worthington KH, Brice AK, Marx JO, Hankenson FC.** Intraperitoneal Injection of Ethanol for the Euthanasia of Laboratory Mice (*Mus musculus*) and Rats (*Rattus norvegicus*). *J Am Assoc Lab Anim Sci.* 2015;54(6):769-778.
- 19- Ohkawa H, Ohishi N, Yagi K.** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979;95(2):351-358. doi:10.1016/0003-

- 2697(79)90738-3
- 20- Bancroft, J.D., Gamble, M., 2019.** Theory and practice of histological techniques. eighth edition, London, England, Elsevier, Pp: 121-132.
- 21- Duman RS, Deyama S, Fogaça MV.** Role of BDNF in the pathophysiology and treatment of depression: Activity-dependent effects distinguish rapid-acting antidepressants. *Eur J Neurosci.* 2021;53(1):126-139. doi:10.1111/ejn.14630
- 22- Lesse A, Rether K, Gröger N, Braun K, Bock J.** Chronic Postnatal Stress Induces Depressive-like Behavior in Male Mice and Programs second-Hit Stress-Induced Gene Expression Patterns of OxtR and AvpR1a in Adulthood. *Mol Neurobiol.* 2017;54(6):4813-4819. doi:10.1007/s12035-016-0043-8
- 23- Beurel E, Toups M, Nemeroff CB.** The Bidirectional Relationship of Depression and Inflammation: Double Trouble. *Neuron.* 2020;107(2):234-256. doi:10.1016/j.neuron.2020.06.002
- 24- Thompson SM, Kallarackal AJ, Kvarita MD, Van Dyke AM, LeGates TA, Cai X.** An excitatory synapse hypothesis of depression. *Trends Neurosci.* 2015;38(5):279-294. doi:10.1016/j.tins.2015.03.003
- 25- Zhang JC, Yao W, Dong C, et al.** Prophylactic effects of sulforaphane on depression-like behavior and dendritic changes in mice after inflammation [published correction appears in *J Nutr Biochem.* 2021 Feb;88:108550] [published correction appears in *J Nutr Biochem.* 2021 Mar;89:108562]. *J Nutr Biochem.* 2017;39:134-144. doi:10.1016/j.jnutbio.2016.10.004
- 26- Sen S, Duman R, Sanacora G.** Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. *Biol Psychiatry.* 2008;64(6):527-532. doi:10.1016/j.biopsych.2008.05.005
- 27- Li MX, Li Q, Sun XJ, et al.** Increased Homer1-mGluR5 mediates chronic stress-induced depressive-like behaviors and glutamatergic dysregulation via activation of PERK-eIF2 α . *Prog Neuropsychopharmacol Biol Psychiatry.* 2019;95:109682. doi:10.1016/j.pnpbp.2019.109682
- 28- Malberg JE, Blendy JA.** Antidepressant action: to the nucleus and beyond. *Trends Pharmacol Sci.* 2005;26(12):631-638. doi:10.1016/j.tips.2005.10.005
- 29 - de Sousa CN, Meneses LN, Vasconcelos GS, et al.** Reversal of corticosterone-induced BDNF alterations by the natural antioxidant alpha-lipoic acid alone and combined with desvenlafaxine: Emphasis on the neurotrophic hypothesis of depression. *Psychiatry Res.* 2015;230(2):211-219. doi:10.1016/j.psychres.2015.08.042
- 30- Liao L, Zhang XD, Li J, et al.** Pioglitazone attenuates lipopolysaccharide-induced depression-like behaviors, modulates NF- κ B/IL-6/STAT3, CREB/BDNF pathways and central serotonergic neurotransmission in mice. *Int Immunopharmacol.* 2017;49:178-186. doi:10.1016/j.intimp.2017.05.036

- 31- Greger IH, Watson JF, Cull-Candy SG.** Structural and Functional Architecture of AMPA-Type Glutamate Receptors and Their Auxiliary Proteins. *Neuron*. 2017;94(4):713-730. doi:10.1016/j.neuron.2017.04.009
- 32- Carvalho AL, Caldeira MV, Santos SD, Duarte CB.** Role of the brain-derived neurotrophic factor at glutamatergic synapses. *Br J Pharmacol*. 2008;153 Suppl 1(Suppl 1):S310-S324. doi:10.1038/sj.bjp.0707509
- 2018;9(1):4272. Published 2018 Oct 15. doi:10.1038/s41467-018-06675-3
- 33-Caldeira MV, Melo CV, Pereira DB, et al.** Brain-derived neurotrophic factor regulates the expression and synaptic delivery of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunits in hippocampal neurons [published correction appears in *J Biol Chem*. 2007 Sep 14;282(37):27556]. *J Biol Chem*. 2007;282(17):12619-12628. doi:10.1074/jbc.M700607200
- 34-Zhang H, Zhang C, Vincent J, et al.** Modulation of AMPA receptor surface diffusion restores hippocampal plasticity and memory in Huntington's disease models. *Nat Commun*
- 35- Ruiz NAL, Del Ángel DS, Olguín HJ, Silva ML.** Neuroprogression: the hidden mechanism of depression. *Neuropsychiatr Dis Treat*. 2018;14:2837-2845. Published 2018 Oct 30. doi:10.2147/NDT.S177973
- 36- Zhao J, Bi W, Xiao S, et al.** Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice. *Sci Rep*. 2019;9(1):5790. Published 2019 Apr 8. doi:10.1038/s41598-019-42286-8
- 37- Li W, Yang S, Kim SO, Reid G, Challis JR, Bocking AD.** Lipopolysaccharide-Induced Profiles of Cytokine, Chemokine, and Growth Factors Produced by Human Decidual Cells Are Altered by *Lactobacillus rhamnosus* GR-1 Supernatant. *Reprod Sci*. 2014;21(7):939-947. doi:10.1177/1933719113519171
- 38- Wang H, Sama AE.** Anti-inflammatory role of fetuin-A in injury and infection. *Curr Mol Med*. 2012;12(5):625-633. doi:10.2174/156652412800620039
- 39- Kim J, Kwak HJ, Cha JY, et al.** Metformin suppresses lipopolysaccharide (LPS)-induced inflammatory response in murine macrophages via activating transcription factor-3 (ATF-3) induction. *J Biol Chem*. 2014;289(33):23246-23255. doi:10.1074/jbc.M114.577908
- 40-Lucca G, Comim CM, Valvassori SS, et al.** Increased oxidative stress in submitochondrial particles into the brain of rats submitted to the chronic mild stress paradigm. *J Psychiatr Res*. 2009;43(9):864-869. doi:10.1016/j.jpsychires.2008.11.002
- 41- Lindqvist D, Dhabhar FS, James SJ, et al.** Oxidative stress, inflammation and treatment response in major depression. *Psychoneuroendocrinology*. 2017;76:197-205. doi:10.1016/j.psyneuen.2016.11.031
- 42- Asadi E, Najafi A, Moeini A, et al.** Ovarian tissue culture in the presence of VEGF and fetuin stimulates follicle growth and steroidogenesis. *J Endocrinol*. 2017;232(2):205-219. doi:10.1530/JOE-16-0368

- 43- Mukhuty A, Fouzder C, Kundu R.** Fetuin-A excess expression amplifies lipid induced apoptosis and β -cell damage. *J Cell Physiol.* 2022;237(1):532-550. doi:10.1002/jcp.30499
- 44- Frenois F, Moreau M, O'Connor J, et al.** Lipopolysaccharide induces delayed FosB/DeltaFosB immunostaining within the mouse extended amygdala, hippocampus and hypothalamus, that parallel the expression of depressive-like behavior. *Psychoneuroendocrinology.* 2007;32(5):516-531. doi:10.1016/j.psyneuen.2007.03.005
- 45- Gassen NC, Rein T.** Is There a Role of Autophagy in Depression and Antidepressant Action? *Front Psychiatry.* 2019; 10:337. Published 2019 May 15. doi:10.3389/fpsyt.2019.00337
- 46- Soares ROS, Losada DM, Jordani MC, Évora P, Castro-E-Silva O.** Ischemia/Reperfusion Injury Revisited: An Overview of the Latest Pharmacological Strategies. *Int J Mol Sci.* 2019;20(20):5034. Published 2019 Oct 11. doi:10.3390/ijms20205034
- 47- Rubab S, Naeem K, Rana I, et al.** Enhanced neuroprotective and antidepressant activity of curcumin-loaded nanostructured lipid carriers in lipopolysaccharide-induced depression and anxiety rat model. *Int J Pharm.* 2021;603:120670. doi:10.1016/j.ijpharm.2021.120670
- 48- Khan MS, Ali T, Abid MN, et al.** Lithium ameliorates lipopolysaccharide-induced neurotoxicity in the cortex and hippocampus of the adult rat brain. *Neurochem Int.* 2017;108:343-354. doi:10.1016/j.neuint.2017.05.008
- 49- Chung HS, Choi KM.** Organokines in disease. *Adv Clin Chem.* 2020;94:261-321. doi:10.1016/bs.acc.2019.07.012