

Brain Derived Neurotrophic Factor has A Role in the Antidepressant Effect of Atorvastatin and Fenofibrate in Wistar Rats

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Abstract

This research aims to investigate the potential antidepressant properties of atorvastatin (ATV), fenofibrate (FFB) and their combination and compare them with fluoxetine (FLX) in the chronic mild stress (CMS) model of depression. Adult male wistar rats were assigned at random into 6 groups (n=8). Control group: received Dimethyl sulfoxide (DMSO 0.5%. p.o). CMS-group: received (DMSO 0.5%. p.o). FLX-treated group: received FLX (10 mg/kg/day. p.o). ATV-treated group: received ATV (10 mg/kg/day. p.o). FFB-treated group: received FFB (200 mg/kg/day. p.o). ATV+FFB-treated group: received combination of ATV and FFB. All groups received treatments 24hrs, 5 hrs and 1 h before OFT and AFST. Then after a 14-day washout period, all groups were given the same treatments and exposed to CMS (except control group) for 3 successive weeks. One hour after the last dose, OFT and CFST were conducted. The data showed that the immobility time in CFST, brain MDA, and IL6 levels were significantly elevated in the CMS group than in the control group. While, brain SOD, BDNF, SE and DA levels, were significantly reduced. In contrast to the CMS group, treatment with FLX, ATV, FFB and the combination of ATV and FFB resulted in a significant reduction in immobility time, MDA, and IL6 levels, as well as a significant increase in SOD, BDNF, SE and DA levels except FFB group that showed non-significant difference with CMS group in DA level. In conclusion, ATV, FFB and their combination presented antidepressant-like effect in CMS-induced depression in male rats.

Keywords: Atorvastatin; Fenofibrate; Depression; BDNF; IL 6.

1. Introduction

Depression is a major contributor to the global disease burden. Although first-line antidepressants are readily available, they have significant limitations, including substantial side effects, delayed therapeutic onset, and limited effectiveness. Antidepressant response rates are between 50 to 60%, and after four therapy steps spread over a year, roughly one-third of depressed patients still have symptoms. Most of the antidepressants

currently prescribed work primarily by altering monoaminergic neurotransmission, which includes serotonin (SE), noradrenaline (NA), and dopamine (DA) (James et al., 2018; De Giorgi et al., 2021). Consequently, there is rising interest in identifying effective and safe medications that might target newly emerging pathophysiological pathways linked to depression as alternative treatments for managing this mental disorder (Ebada, 2017).

Depression has a diverse and complex pathophysiology. The development of depression is strongly influenced by immunological dysfunction and inflammation (Anderson et al., 2014). Peripheral inflammatory cytokine signals are conducted to the CNS through humoral and neuronal pathways, they may produce depression by interacting with the hypothalamic-pituitary-adrenal axis (HPA-axis), impairing glutamate, SE, DA, and NA neurotransmitter systems, reducing neurogenesis, interfering with mitochondrial biogenesis, intensifying oxidative stress, and producing long-lasting and damaging brain alterations (Fernandez-Sanchez et al., 2011; Kim et al., 2019).

Brain-derived neurotrophic factor (BDNF) is one of the neurotrophic factors that provoke synaptogenesis, and neuronal proliferation as well as being involved in the control of neurogenesis (Kosowski et al., 2021). It has been hypothesized that BDNF signaling pathway plays a crucial role in the pathophysiology of depression (Jeon et al., 2016). Serum BDNF is reduced in depressed individuals and adversely connected with depression severity (Lee et al., 2007). Furthermore, it has been demonstrated that BDNF expression is enhanced following antidepressant therapy (Martinowich et al., 2007).

Atorvastatin (ATV) is a lipophilic and synthetic statin, primarily used to treat hypercholesterolemia. ATV acts by inhibiting the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis (Wang et al., 2011). Clinical trials proved that statins use lowers the risk of stroke (Di Napoli et al., 2002) and dementia (Stépieň et al., 2002). The neuroprotective effects of statins have also been shown in research using animal models (Ouk et al., 2014b) and cell cultures (Posada-Duque et al., 2013).

Anti-inflammatory, antioxidant, neurotrophic, and possibly monoamine-based actions may all have a role in the potential antidepressant mechanisms of statins (Ludka et al., 2014; Köhler-Forsberg et al., 2017). The statins' anti-inflammatory effects are rapid and unrelated to their capacity to decrease cholesterol (De Giorgi et al., 2021). According to animal studies, statins lessen depressive-like symptoms by lowering hippocampal neuroinflammation (Wu et al., 2019). Moreover, statins function as ligands for the nuclear receptor, peroxisome proliferator-activated receptor α

(PPAR α), whose activation boosts cyclic AMP response element-binding protein (CREB) transcription and promotes the expression of neurotrophic factors such as BDNF and neurotrophin-3 (NT-3) (Roy et al., 2015).

Fenofibrate (FFB), a fibric acid derivative, is used to treat adults with primary hypercholesterolemia, mixed dyslipidemia, and hypertriglyceridemia (Keating and Croom, 2007). More pharmacological effects of FFB on the CNS are found, including its capacity to maintain adult hippocampal neurogenesis, its neuroprotective properties against Parkinson's disease, and its ability to inhibit memory impairments after global cerebral ischemia in rats (Barbiero et al., 2014a; Ouk et al., 2014a).

Additionally, FFB is a selective agonist of PPAR α , in which this receptor and its endogenous ligands, e.g., oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), regulate energy homeostasis and modulate neuroinflammation, neurogenesis, glial cell proliferation/differentiation, antioxidant responses, and affect neurotransmission as well (Wójtowicz et al., 2020). Therefore, PPAR α agonists may provide protection against neuropsychiatric illnesses and neurodegenerative diseases (Scheggi et al., 2022). FFB treatment suppressed depression-like characteristics in male mice to a degree comparable to fluoxetine (FLX). The activation of PPAR α - mediated enhancement of the hippocampus BDNF signaling cascade and higher production of BDNF were linked to the protective effect (Jiang et al., 2017).

Therefore, this study was conducted to explore the potential antidepressant properties of ATV, FFB and their combination in chronic mild stress (CMS)-induced depression and to evaluate their effect on BDNF, proinflammatory cytokines, oxidative stress parameters, and monoamine neurotransmitters in the brain of CMS induced mice. This was done on the basis of good tolerability, general safety, and widespread use of statins and fibrates.

2. Materials and Methods

2.1. Drugs and Chemicals

Fluoxetine hydrochloride, atorvastatin (calcium salt trihydrate) and fenofibrate were purchased from AK Scientific, Inc. (USA).

Brain derived neurotrophic factor (BDNF) and Interleukin 6 (IL6) assay kits were purchased from

Elabscience Biotechnology, Inc. (USA). Kits from Bio-diagnostic Company, Egypt, were used to measure superoxide dismutase (SOD) and malondialdehyde (MDA). Cusabio Technology LLC (USA) provided kits for measuring SE and DA.

2.2. Animals

This study was performed on 48 adult male wistar rats (average weight 230-250g) that were taken from the animal house and housed in the animal facility at the Faculty of Medicine at Sohag University. The rats were housed in a controlled environment with a standard temperature of (25±2°C). 12 hours of light followed by 12 hours of darkness were delivered by a time-controlled system under constant environmental conditions (55±10% humidity). All rats had unlimited access to rodent chow food and water.

The experimental protocol was carried out and approved according to the guidelines of the Medical Research Ethics Committee of Faculty of Medicine, Sohag University, Egypt (Approval No. Soh-Med-21-12-37).

2.3. Experimental design

Animals were assigned randomly into sex groups with eight animals each after acclimatization for a week.

Control group: rats received Dimethyl sulfoxide (DMSO 0.5% p.o).

CMS group: rats received Dimethyl sulfoxide (DMSO 0.5% p.o).

FLX-treated group: rats received FLX (10 mg/kg p.o) which used as a positive control (**Ludka et al., 2014**).

ATV-treated group: rats received ATV (10 mg/kg p.o) (**Taniguti et al., 2019**).

FFB-treated group: rats of this group received FFB (200 mg/kg p.o) (**Ouk et al., 2014a**).

ATV+FFB-treated group: rats of this group received combination of ATV (10 mg/kg p.o) and FFB (200 mg/kg p.o).

On the first day of the experiment, all groups were permitted to swim for 15 minutes in the water. Then, all groups got either the vehicle or the drugs 24 hrs, 5hrs and 1h before open field test (OFT) and

acute forced swim test (AFST) were done.

Then, all groups were given their treatments once a day for 21 days after a 14-day washout period, and were exposed to CMS (except control group) to induce chronic depression. On day 21, one hour after the last dose, OFT and chronic forced swim test (CFST) were conducted.

2.3.1. Chronic mild stress (CMS) procedure

The CMS procedure was carried out in accordance with **Willner's (1997)** method with minor modifications. These stress techniques briefly consists of the following: 1) a 24-h fast from food, 2) 24 hrs without water or a drink container, 3) cage slanted at a 45° angle for 24 hrs, 4) kept together as a group for 2 hrs before being divided into individual cages, 5) application of restraining stress for 2 hrs, 6) lighting for 12 hrs at night, 7) tail clamping for 15 minutes, 8) forced cold water swimming (4°C–8°C) for 5 minutes, 9) being exposed to a new environment for 24 hrs, and 10) an empty water bottle. The rats were subjected to one of the aforementioned stress methods at random every day for three weeks consecutively; the same technique was not used repeatedly to prevent the rats from developing a sense of anticipation for the upcoming stress method.

2.4. Behavioral tests

2.4.1. Forced Swim Test (FST)

2.4.1.1. Acute Forced Swim Test (AFST)

Experiments were carried out according to the method of **Porsolt et al., (1978)**. A glass cylindrical water tank with a 20 cm diameter and a height of 45 cm was employed.

This test was conducted in two sessions:

Pretest Session: This test was conducted a day before the real test. Warm water (25 °C) was poured into the glass tank until it was 15 cm high. In the tank, each rat was put separately, and they were all given 15 minutes to swim. Rats were removed from their cages following the pretest and then cleaned, dried, and placed back inside their own cages.

Test Session: The rats were subjected to the same circumstances as before, and by using a stopwatch, the total time of immobility (measured in seconds)

over a period of five minutes was recorded. They initially fought to get out of the water, but eventually settled into a position of immobility, making only the movements required to maintain their heads above water. When a rat floated upright in the water, moving only slightly to keep its head above water, it was considered immobile.

2.4.1.2. Chronic Forced Swim Test (CFST)

Animals used in AFST were used in this test. They received their treatments for 21 days. One hour after the last dose was given, they underwent FST for 5 minutes. Each animal's immobility time was recorded throughout this time.

2.4.2. Open Field test (OFT)

To measure the animals' locomotor activity, OFT was performed five minutes before the acute or chronic FST. Animals given the study medicines were monitored for activity in an open field to see whether a change in immobility is connected to a change in motor activity. A wooden box measuring 60 cm X 60 cm with 30 cm high walls served as the apparatus for the OFT. There were nine equal-sized smaller squares on its floor (20 cm X 20 cm). Using manually controlled counters, the number of line crossings, rearing, and grooming frequencies were recorded. The behavioral parameters of each rat were monitored for 5 minutes while it was positioned in the center of the arena. Before housing the subsequent animal, the equipment was cleaned with a detergent solution to get rid of any odor left by the previous animal (Vogel, 2002).

2.5. Sample collection

All groups of rats underwent ether anesthesia after all behavioral tests were completed. Animals were sacrificed thereafter by cervical dislocation to separate tissue samples. Each animal's brain was quickly removed. Then, tissue samples were taken from them to measure the levels of IL6, SOD, MDA, BDNF, SE and DA.

2.6. Biochemical analysis

2.6.1. Measurements of IL6, SOD, and MDA

After being rinsed in ice cold saline, a piece of the brain was weighed and homogenized in phosphate buffered saline (pH 7.4) using a homogenizer. After centrifuging the homogenate, the supernatant was removed and stored at -80°C until IL6, SOD, and MDA were measured.

Interleukin 6 (IL6) was measured in brain tissue using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. The IL6 was expressed as pg/g tissue.

Superoxide Dismutase (SOD) was assayed in brain tissue by a colorimetric method according to the method of Nishikimi et al., (1972). The level of SOD was expressed in U/g tissue.

Malondialdehyde (MDA) was estimated in brain tissue by a colorimetric method according to the method of Ohkawa et al., (1979). MDA level was expressed in nmol/g tissue.

2.6.2. Measurements of BDNF, SE, and DA

After homogenization and centrifugation of the brain tissue, the supernatant was also used to assay BDNF, SE, and DA levels.

Brain Derived Neurotrophic Factor (BDNF), SE, and DA were assayed in brain tissue using ELISA according to the manufacturer's instructions. The BDNF was expressed as pg/g tissue. while SE and DA were expressed as ng/g tissue.

2.7. Statistical analysis of data

Values were expressed as mean \pm SE. The data were analysed using SPSS software (Statistical Package for the Social Sciences, version 25.0, SPSS Inc, Chicago, IL), and a one-way analysis of variance (ANOVA). Tukey's post hoc test was used to compare the groups' mean values. $P < 0.05$ was considered statistically significant in all types of statistical tests.

3. Results

3.1. Acute Forced Swim Test (AFST)

Figure (1) shows that the treatment of rats with FLX (10 mg/kg p.o), ATV (10 mg/kg p.o), FFB (200 mg/kg p.o), and the combination of ATV and FFB 24hrs, 5 hrs and 1 h before the test decreased the duration of immobility significantly ($P < 0.05$) compared to the control and CMS group.

3.2. Open Field Test (OFT) for AFST

The OFT (Table 1) shows a non-significant difference ($P > 0.05$) in the frequency of line crossings, rearings, and groomings when drug-treated groups were compared to rats in the control and CMS groups, excluding any general stimulant or depressive action.

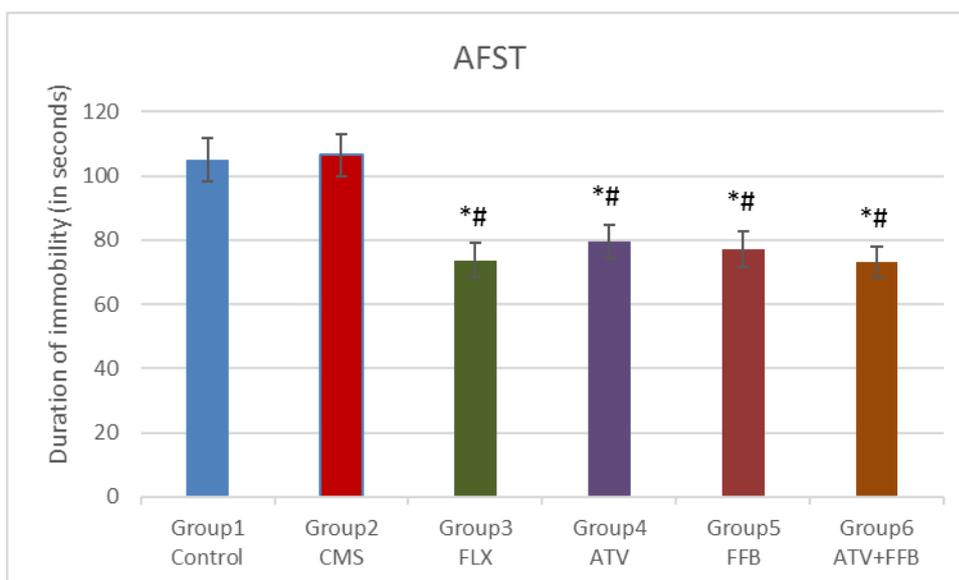


Figure 1. Duration of immobility in rats subjected to Acute Forced Swim Test (AFST). The data were analysed using a one-way ANOVA followed by a Tukey post hoc test and the values represent mean \pm SEM (n=8). (*) means significantly ($P < 0.05$) different from the control group, and (#) means significantly ($P < 0.05$) different from the CMS group. CMS=Chronic mild stress, FLX=Fluoxetine, ATV=Atorvastatin, FFB= Fenofibrate.

Table 1. Locomotor activity parameters in Open Field Test for AFST

Groups	Crossing	Rearing	Grooming
Group 1: control	28.50 \pm 1.25	8.00 \pm 0.53	2.25 \pm 0.16
Group 2: CMS	28.88 \pm 1.64	7.75 \pm 0.36	2.00 \pm 0.18
Group 3: FLX	30.25 \pm 1.89	8.63 \pm 0.49	2.13 \pm 0.12
Group 4: ATV	27.75 \pm 1.34	7.63 \pm 0.37	2.5 \pm 0.18
Group 5: FFB	29.63 \pm 1.47	7.88 \pm 0.51	2.25 \pm 0.16
Group 6: ATV+FFB	27.38 \pm 1.65	8.38 \pm 0.68	2.38 \pm 0.18

The data were analysed using a one-way ANOVA followed by a Tukey post hoc test and the values represent mean \pm SEM (n=8). There is a non-significant ($P > 0.05$) difference between different drug-treated groups and control and CMS groups. CMS=Chronic mild stress, FLX=Fluoxetine, ATV=Atorvastatin, FFB= Fenofibrate.

3.3. Chronic Forced Swim Test (CFST)

Figure (2) demonstrates that the duration of immobility increased significantly ($p < 0.05$) in the CMS group compared to the control group, while the duration of immobility decreased significantly ($P < 0.05$) in the groups of rats received FLX, ATV, FFB and the combination of ATV and FFB for 21-days compared to the CMS group, with non-statistically significant ($P > 0.05$) difference between the drug groups and the control group and between different drug groups.

3.4. Open Field Test (OFT) for CFST

Table (2) indicates a significant decrease ($P < 0.05$) in the number of crossings, rearings, and groomings in the CMS group compared to the control group, while there is a non-significant difference ($P > 0.05$) in the number of crossings, rearings, and groomings among drug-treated groups and rats in the control and CMS groups in the OFT. These data indicate that treatment with FLX, ATV, FFB or the combination of ATV and FFB for 21 days did not cause significant increase in locomotor activity.

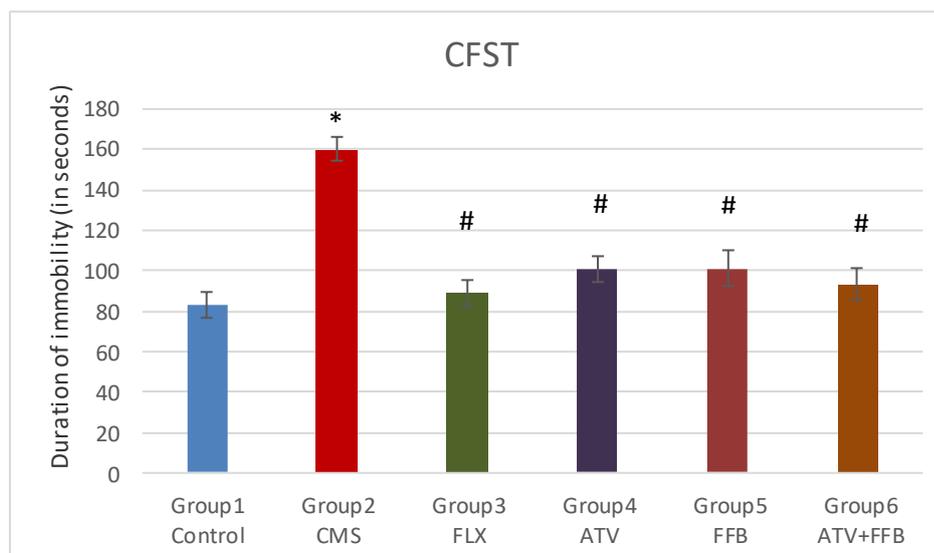


Figure 2. Duration of immobility in rats subjected to Chronic Forced Swim Test (CFST). The data were analysed using a one-way ANOVA followed by a Tukey post hoc test and the values represent mean \pm SEM (n=8). (*) means significantly ($P < 0.05$) different from the control group, and (#) means significantly ($P < 0.05$) different from the CMS group. CMS=Chronic mild stress, FLX=Fluoxetine, ATV=Atorvastatin, FFB=Fenofibrate.

Table 2. Locomotor activity parameters in Open Field Test for CFST

Groups	Crossing	Rearing	Grooming
Group 1: control	25.38 \pm 1.93	8.25 \pm 0.45	3.25 \pm 0.25
Group 2: CMS	17.63 \pm 0.56*	6.25 \pm 0.36*	2 \pm 0.18*
Group 3: FLX	24.38 \pm 2.19	7.63 \pm 0.42	2.88 \pm 0.22
Group 4: ATV	23.25 \pm 2.05	7.5 \pm 0.42	3.00 \pm 0.26
Group 5: FFB	24.13 \pm 1.72	7.88 \pm 0.39	2.75 \pm 0.25
Group 6: ATV+FFB	22.75 \pm 1.59	7.75 \pm 0.36	3.00 \pm 0.26

The data were analysed using a one-way ANOVA followed by a Tukey post hoc test and the values represent mean \pm SEM (n=8). (*) means significantly ($P < 0.05$) different from the control group. CMS=Chronic mild stress, FLX=Fluoxetine, ATV=Atorvastatin, FFB=Fenofibrate.

3.5. Effect of FLX, ATV, FFB, and the combination of ATV and FFB on brain SOD level

Figure (3) demonstrates a significant decrease ($P < 0.05$) in the brain SOD level in the CMS group compared to the control group, while 21-day administration of FLX, ATV, FFB and the combined administration of ATV and FFB produced a significant increase ($P < 0.05$) in brain SOD activity compared to the CMS animals. Furthermore, these four groups of drugs show a

non-statistically significant ($P > 0.05$) difference between each other and between them and the control group.

3.6. Effect of FLX, ATV, FFB, and the combination of ATV and FFB on brain MDA level

As shown in **figure (4)**, there is a significant increase ($P < 0.05$) in the brain MDA level in the CMS group compared to the control group, while the administration of FLX, ATV, FFB and the combination of ATV and FFB for 21-days led to a

significant decrease ($P < 0.05$) in brain MDA activity compared to the CMS group. Additionally, these four groups of drugs show non-statistically

significant ($P > 0.05$) difference between each other, and between them and the control group.

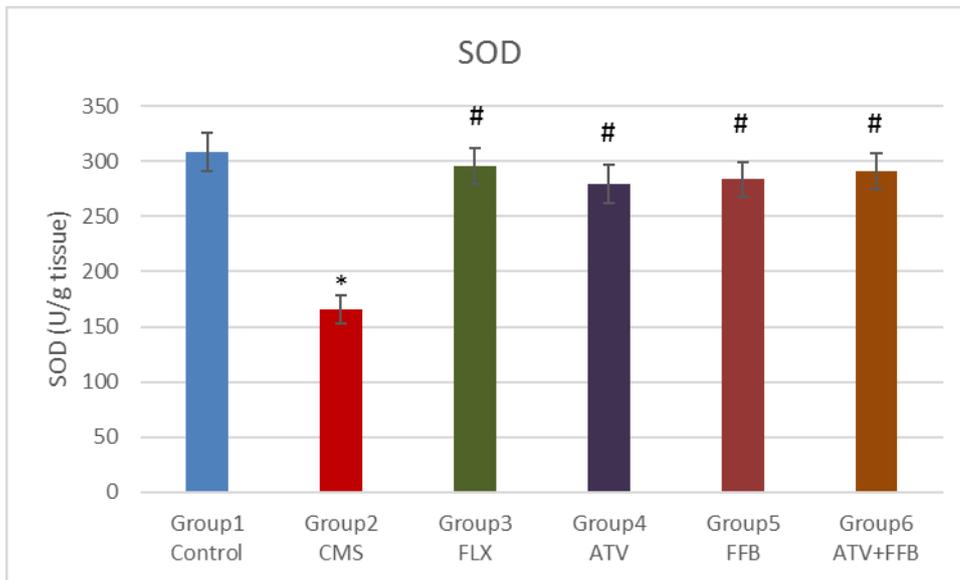


Figure 3. Effect of fluoxetine (10 mg/kg/day p.o), atorvastatin (10 mg/kg/day p.o), fenofibrate (200 mg/kg/day p.o), and the combination of ATV and FFB on brain SOD level in chronic mild stress-induced depression in Wistar rats. The data were analysed using a one-way ANOVA followed by a Tukey post hoc test and the values represent mean \pm SEM (n=8). (*) means significantly ($P < 0.05$) different from the control group, and (#) means significantly ($P < 0.05$) different from the CMS group. CMS=Chronic mild stress, FLX=Fluoxetine, ATV=Atorvastatin, FFB= Fenofibrate, SOD=superoxide dismutase.

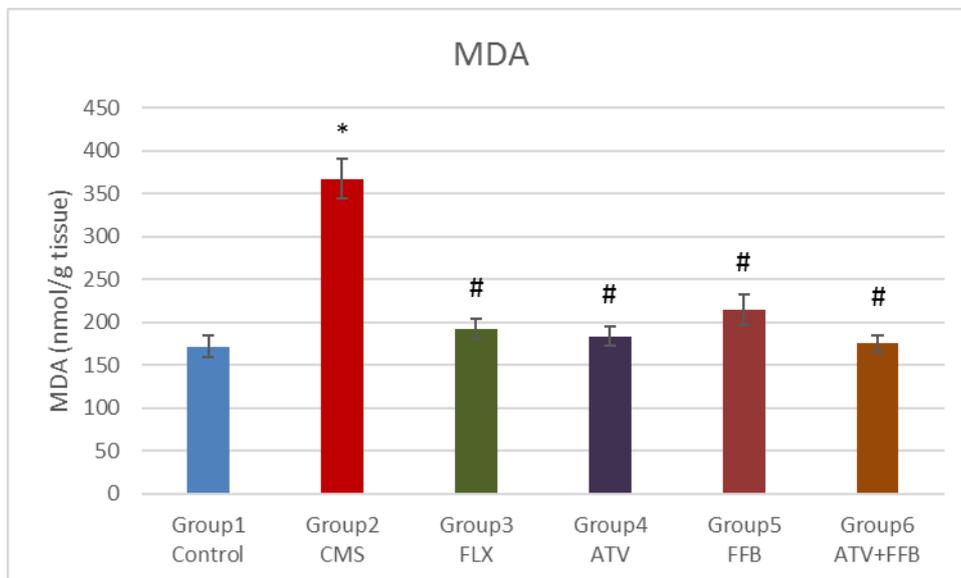


Figure 4. Effect of fluoxetine (10 mg/kg/day p.o), atorvastatin (10 mg/kg/day p.o), fenofibrate (200 mg/kg/day p.o), and the combination of ATV and FFB on brain MDA level in chronic mild stress-induced depression in Wistar rats. The data were analysed using a one-way ANOVA followed by a Tukey post hoc test and the values represent mean \pm SEM (n=8). (*) means significantly ($P < 0.05$) different from the control group, and (#) means significantly ($P < 0.05$) different from the CMS group. CMS=Chronic mild stress, FLX=Fluoxetine, ATV=Atorvastatin, FFB= Fenofibrate, MDA=Malondialdehyde.

3.7. Effect of FLX, ATV, FFB, and the combination of ATV and FFB on brain IL6 level

Figure (5) reveals a significant increase ($P < 0.05$) in the brain IL6 level in the CMS group compared to the control group, while the treatment of rats with FLX, ATV, FFB and the combination of ATV and FFB for 3 successive weeks caused a significant decrease ($P < 0.05$) in brain IL6 activity relative to the CMS group. Also, these four groups of drugs showed a non-statistically significant ($P > 0.05$) difference between each other, and between them and the control group.

3.8. Effect of FLX, ATV, FFB, and the combination of ATV and FFB on brain BDNF level

As demonstrated in **figure (6)**, there is a significant decrease ($P < 0.05$) in the brain BDNF level in the CMS group compared to the control group. However, compared to the CMS animals, 21-day administration of FLX, ATV, FFB and the combination of ATV and FFB produced a significant increase ($P < 0.05$) in brain BDNF activity. Furthermore, these four groups of drugs show non-statistically significant ($P > 0.05$) difference between each other, and between them and the control group.

3.9. Effect of FLX, ATV, FFB, and the combination of ATV and FFB on brain monoamine neurotransmitters (SE, DA)

Table (3) indicates a significant decrease ($P < 0.05$) in the brain SE and DA levels in the CMS group compared to the control group. In contrast to the CMS group, treatment with FLX, ATV, and the combination of ATV and FFB for 3 consecutive weeks led to a significant increase ($P < 0.05$) in the brain SE activity. The FFB-treated group showed a non-statistically significant ($P > 0.05$) difference from the CMS group and a significant decrease ($P < 0.05$) in the brain SE level compared to the control and FLX-treated groups. Additionally, FLX, ATV, and the combination of ATV and FFB show non-statistically significant ($P > 0.05$) difference in the brain SE level between each other, and between them and the control group.

Furthermore, the 21-day administration of FLX, ATV, FFB, and the combined administration of ATV and FFB produced a significant increase

($P < 0.05$) in the brain DA activity compared to the CMS animals, while the four drug groups show non-statistically significant ($P > 0.05$) difference between each other, and between them and the control group.

4. Discussion

Depression is a common disabling disorder (**James et al., 2018**) for which existing treatment options, such as psychotherapy and pharmacotherapy, are not satisfactory (**De Giorgi et al., 2022**). Therefore, it is essential to find new molecular targets for depression and new medicines acting on those targets (**Jarończyk and Walory, 2022**).

The present study demonstrates that behavioral changes induced by CMS in the FST could be avoided by ATV and FFB. Moreover, ATV and FFB antidepressant-like effect was associated with the prevention of changes induced by CMS on IL6 level, oxidative stress, BDNF, and monoamine neurotransmitters (SE, DA) level in the brain.

Models for forced swim test were selected as they are frequently used to evaluate antidepressant medications (**Devadoss et al., 2010**). The findings of our study revealed that administration of FLX, ATV, FFB, or the combination of ATV+FFB 24hrs, 5 hrs and 1 h before AFST caused a significant decrease in the duration of immobility when compared with the control group and CMS group. Our results are consistent with **Ludka et al. (2013)**, who showed that acute ATV administration reduced the immobility time in FST. In addition, **Jiang et al. (2017)** reported that a single injection of FFB and FLX induced a decrease in immobility time in the FST. Our study also indicated that the CMS group showed a significant increase in the duration of immobility in CFST which agrees with the previous report (**Song et al., 2020**). However, administration of FLX, ATV, FFB, and the combined treatment of ATV and FFB for 21 days caused a significant decrease in the duration of immobility when compared with the CMS group, which indicates that ATV and FFB have an antidepressant-like effect. This is in line with **Taniguti et al. (2019)** who showed that ATV or FLX treatment prevented lipopolysaccharide (LPS) induced increase in immobility time. Furthermore, **Barbiero et al. (2014b)** showed that a significant reduction in immobility was observed in the FFB-treated group in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced model of parkinsonism.

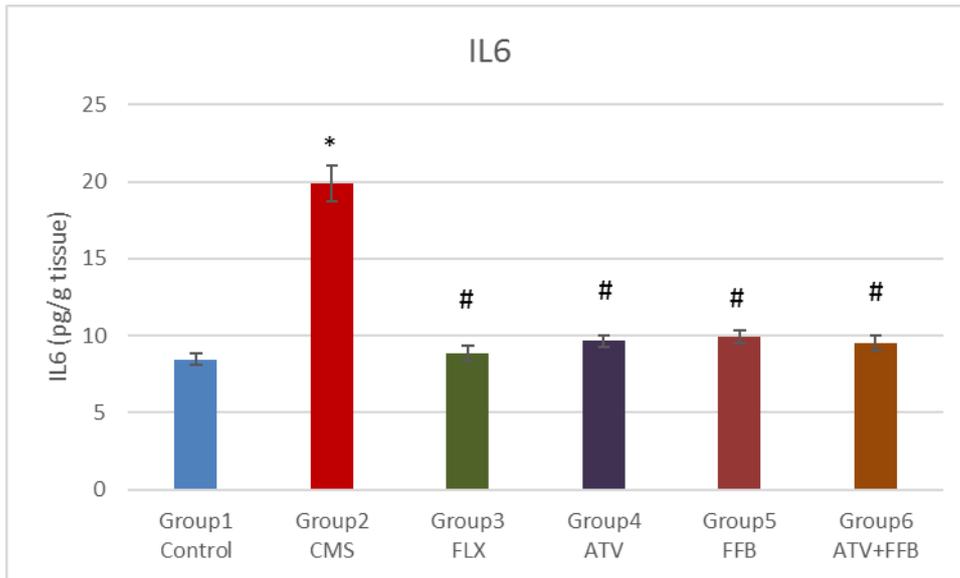


Figure 5. Effect of fluoxetine (10 mg/kg/day p.o), atorvastatin (10 mg/kg/day p.o), fenofibrate (200 mg/kg/day p.o), and the combination of ATV and FFB on brain IL6 level in chronic mild stress-induced depression in Wistar rats. The data were analysed using a one-way ANOVA followed by a Tukey post hoc test and the values represent mean \pm SEM (n=8). (*) means significantly ($P < 0.05$) different from the control group, and (#) means significantly ($P < 0.05$) different from the CMS group. CMS=Chronic mild stress, FLX=Fluoxetine, ATV=Atorvastatin, FFB= Fenofibrate, IL6=Interleukin 6.

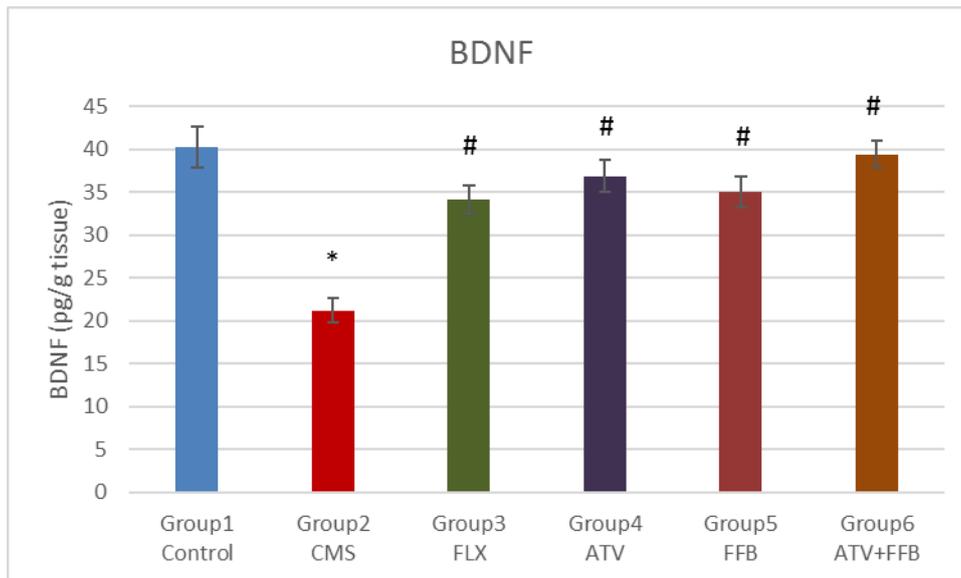


Figure 6. Effect of fluoxetine (10 mg/kg/day p.o), atorvastatin (10 mg/kg/day p.o), fenofibrate (200 mg/kg/day p.o), and the combination of ATV and FFB on brain BDNF level in chronic mild stress-induced depression in Wistar rats. The data were analysed using a one-way ANOVA followed by a Tukey post hoc test and the values represent mean \pm SEM (n=8). (*) means significantly ($P < 0.05$) different from the control group, and (#) means significantly ($P < 0.05$) different from the CMS group. CMS=Chronic mild stress, FLX=Fluoxetine, ATV=Atorvastatin, FFB= Fenofibrate, BDNF=Brain derived neurotrophic factor.

Table 3: Effect of fluoxetine (10 mg/kg/day p.o), atorvastatin (10 mg/kg/day p.o), fenofibrate (200 mg/kg/day p.o), and the combination of ATV and FFB on brain monoamine neurotransmitters (SE, DA) level

Groups	SE ng/g tissue	DA ng/g tissue
Group 1: control	1.38±0.12	0.44±0.04
Group 2: CMS	0.85±0.05*	0.23±0.01*
Group 3: FLX	1.37±0.09 [#]	0.4±0.02 [#]
Group 4: ATV	1.34±0.08 [#]	0.35±0.02 [#]
Group 5: FFB	0.98±0.05* [●]	0.4±0.02 [#]
Group 6: ATV+FFB	1.28±0.08 [#]	0.42±0.03 [#]

The data were analysed using a one-way ANOVA followed by a Tukey post hoc test and the values represent mean \pm SEM (n=8). (*) means significantly ($P < 0.05$) different from the control group, (#) means significantly ($P < 0.05$) different from the CMS group. (●) significantly ($P < 0.05$) different from the FLX group. CMS=Chronic mild stress, FLX=Fluoxetine, ATV=Atorvastatin, FFB=Fenofibrate, SE=Serotonin, DA=Dopamine.

Open field test is typically used to distinguish between the study drugs' antidepressant effects and the general behavioral stimulation (false positives). The current study showed that OFT for AFST showed a non-significant difference in locomotor activity between drug-treated groups and the control and CMS groups. This is in agreement with the previous reports (Ludka et al., 2013; Jiang et al., 2017), while the OFT for CFST showed a significant decrease in locomotor activity in the CMS group compared to the control group. This is consistent with Wu et al. (2021) who showed that mice in CUMS group displayed a significant reduction in locomotor activity in OFT. Moreover, there is a non-significant difference in the locomotor activity among drug-treated groups and the control group and CMS group. This is consistent with the previous reports (Barbiero et al., 2014b; Taniguti et al., 2019) confirmed the idea that the effects shown in FST models are unique to the antidepressant action.

According to earlier research, the pathophysiology of depression may be influenced by elevated oxidative stress (Maurya et al., 2016). Low levels of antioxidants like SOD and high levels of MDA, a byproduct of polyunsaturated fatty acid peroxidation, are found in depressed patients, which indicates increased oxidative damage (Maes et al., 2011; Maurya et al., 2016). This is in accordance with our study that revealed a significant decrease

in SOD and a significant increase in MDA levels in CMS group. However, the 21-day administration of FLX, ATV, FFB, and the combined treatment of ATV and FFB caused a significant increase in brain SOD activity relative to the CMS group. This result is in accordance with Kaviani et al. (2017) and Liu et al. (2021) who indicated increased SOD levels upon ATV pretreatment. Also, our results are in accordance with Deplanque et al. (2003) who reported that 14 days of preventive treatment with FFB in ischemic stroke significantly increase brain Copper/zinc SOD.

Administration of FLX, ATV, FFB, and the combined treatment of ATV and FFB produce a significant reduction in brain MDA activity relative to the CMS group. This is in accordance with Taniguti et al. (2019) who mentioned that ATV or FLX treatment attenuated the deleterious elevation of brain MDA. Statins prevent the expression of protein subunits of Gi-proteins (p22phox and gp91phox) that regulate the NADPH oxidase activity and GTP-ase expression (NADPH activator). This reduces the generation of the most dangerous free radicals, superoxide anion and peroxynitrite, and suppresses the activity of prooxidant enzyme systems (NADPH oxidase, xanthine oxidase, and myeloperoxidase). Statins also increase the expression of antioxidant enzymes (Drinitsina and Zateishchikov, 2005). Therefore, it is possible that the antidepressant-like effects of

ATV are due to the restoration of antioxidant defenses and the prevention of oxidative damage. Furthermore, **Rizq et al. (2022)** stated that FFB significantly reduced the hippocampal MDA, attributed to its PPAR α agonist activity causing suppression of NADPH oxidase activity, and increasing the antioxidant capacity by lowering MDA, and stimulating the expression of SOD, a major antioxidant enzyme (**Olukman et al., 2010; Moran et al., 2014**).

There is mounting evidence that inflammation and immunological dysregulation may be involved in the pathogenesis of depressive illnesses. The cytokine hypothesis states that depressive disorders are associated with increased cytokines production, such as interleukins, TNF- α , and interferon- α and γ (**Catena-Dell'Osso et al., 2013**). In this study, the CMS group showed a significant increase in IL6, while all drug-treated groups showed a significant decrease in brain IL6 activity relative to the CMS group. This is in accordance with **Zhang et al. (2013)** who demonstrated that IL-6 was significantly decreased in the ATV-treated Alzheimer's disease (AD) group. Some of the possible anti-inflammatory effects of statins include lowering levels of C-reactive protein (CRP) and antioxidant activity, inhibiting pro-inflammatory cytokines production by monocytes, blocking the function of antigen-1 leukocytes (LFA-1) thus inhibiting lymphocytes, and T-cell activation block (**Kosowski et al., 2021**). These statins-induced anti-inflammatory and antioxidant actions are possible mechanisms for their effects on various psychiatric diseases.

These results are also in agreement with **Rizq et al. (2022)** who indicated that FFB significantly reduced the hippocampal IL6. PPAR α mediates its anti-inflammatory actions in the CNS by transrepressing the inflammatory transcription factors like nuclear factor kappa-light-chain-enhancer of activated B cells (NF κ B), which in turn inhibits the release of inflammatory cytokines, including TNF- α and interleukins 1 and 6 (**Scheggi et al., 2022**).

Previous research has shown that stress reduces BDNF signalling pathway activity in the hippocampus of mice, and that antidepressants like FLX can repair these pathological abnormalities (**Castrén and Rantamäki, 2010; Razzoli et al., 2011**). This is in accordance with our results that demonstrated a significant decrease in BDNF in CMS group.

Fluoxetine, atorvastatin, fenofibrate, and ATV+FFB-treated groups showed a significant increase

in brain BDNF activity compared to the CMS animals, which is in good agreement with **Taniguti et al. (2019)** who showed that one-week treatment with ATV or FLX significantly increased the BDNF levels in the hippocampus and also **Kaviani et al. (2017)** who indicated that the neuroprotective effect of ATV mediated in part by an increase in BDNF. In addition to their general neuroprotective properties, statins may also enhance hippocampal neuroplasticity, which is particularly linked to the pathophysiology of depression and how well it responds to antidepressant therapy (**Arosio et al., 2021**). This is accomplished by increasing BDNF through direct, tissue plasminogen activator (tPA), and agmatine/imidazoline pathways (**Ludka et al., 2013; Taniguti et al., 2019; De Giorgi et al., 2021; Rahangdale et al., 2021**).

In addition, **Jiang et al. (2017)** reported that FFB restored the reduction in hippocampus BDNF signalling cascade caused by chronic social defeat stress (CSDS). It has been noted that PPAR α activation promotes CREB's transcriptional activity and the production of BDNF and other proteins involved in neuroplasticity (**Song et al., 2018**). Accordingly, the long-term treatment of PPAR α synthetic agonists like FFB can reverse behavioral changes caused by stress and restore the hippocampal BDNF signaling that has been reduced by stress exposure (**Jiang et al., 2017**).

The development of depression is strongly correlated with the decrease of monoamine neurotransmitters, which is involved in HPA-axis hyperactivity (**Pariante and Lightman, 2008; Zhao et al., 2018**). Long-term chronic and social stress can cause HPA axis hyperfunction, this further causes a decrease in monoamine neurotransmitters in the central and peripheral nervous systems, which results in depressive-like symptoms (**Pariante and Lightman, 2008**). This agrees with the significant decrease of SE and DA in CMS group.

Our study also indicated that the 21-day administration of FLX, ATV, FFB, and the combined administration of ATV and FFB caused a significant increase in the brain neurotransmitters (SE and DA) activity relative to the CMS group, except for FFB which showed a significant decrease in SE level compared to the control and FLX group and a non-significant difference compared to CMS group. Such recorded results are in agreement with **Al-Asmari et al. (2017)** who

showed that FLX increased the brain concentration of SE and DA up to a significant level in comparison to the control, while simvastatin increased the brain level of SE but has no effect on DA levels, and **Ludka et al. (2014)** who mentioned that the availability of SE in the synaptic cleft and the interaction with serotonin (5-HT1A and 5-HT2) receptors are both necessary for the antidepressant-like effects of ATV.

Although multiple neurotransmitters are implicated (**Kosowski et al., 2021**), the majority of evidence focuses on SE transmission, which implies that statins' antidepressant effects may be attributable to their ability to increase the availability of the SE precursor, tryptophan (Trp), as a result of indoleamine 2,3-dioxygenase (IDO) enzyme blockade, and overall hippocampal serotonin levels (**De Giorgi et al., 2022**). Also, **Wang et al. (2005)** showed that simvastatin upregulates the expression of dopamine D1 and D2 receptors in the rat prefrontal cortex, and **Ghosh et al. (2009)**, stated that simvastatin and pravastatin protected the dopaminergic neurons from death and the related reduction in neurotransmitter levels in MPTP-treated mice.

The current study is also in accordance with **Ohta et al. (2009)** who showed a significant lowering of blood levels of SE and an increase in the kynurenine pathway after fenofibrate treatment for 14 days in a high dose (300mg/kg/day). Additionally, **Ni et al. (2018)** showed that PCPA, a tryptophan hydroxylase inhibitor, did not block the reversal effects of gemfibrozil (PPAR α agonist) on the stressed mice in the FST. Just a small portion of the Trp pool is used for SE biosynthesis. Most Trp in mammals enters the kynurenic route and is transformed into bioactive compounds other than SE, including kynurenic acid and quinolinic acid (**Le Floch et al., 2011**). Fibrate activation of several kynurenine pathway enzymes has been reported previously (**Shin et al., 2006**).

Also, **Barbiero et al. (2014b)** showed that FFB prevented the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the subsequent decrease in the striatum DA levels. Regulation of dopamine neuronal firing in the ventral tegmental area (VTA) is a primary mechanism through which PPAR α may be implicated in the control of major depression and anhedonia. Long-term activation of PPAR α caused by FFB administration may reduce the negative modulatory influence on β 2 subunit-containing nicotinic acetylcholine receptors (β 2nAChRs), thus

reducing the inhibition of VTA dopamine neurons and increasing DA release at levels high enough to trigger transmission in D1 dopamine receptor (**Scheggi et al., 2022**). On these basis, it has been suggested that activation of PPAR α may provoke antidepressant-like effects.

5. Conclusion

In conclusion, the results of this study demonstrated that ATV, FFB and their combination presented antidepressant-like effect in CMS-induced depression in male rats. However, the combination of both drugs showed slightly favorable results over their individual use. The antidepressant effect of ATV and FFB is comparable to FLX which is used as a standard drug for the treatment of depression. So, ATV or FFB may be recommended in patients with hyperlipidemia concomitant with depression or in patients with higher levels of peripheral cytokines who are less likely to respond to antidepressants. ATV and FFB antidepressant-like effect was mediated through decreasing MDA and IL6 levels, and increasing SOD, BDNF, SE, and DA levels in the rat brain. Our results raise the perspective for further investigation of behavioral alterations and molecular mechanisms involved in ATV and FFB antidepressant-like effects.

Conflicts of Interest

No conflicts of interest are disclosed by the authors.

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