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Fluoxetine Ameliorates the behavioral and inflammatory changes induced by Repeated Exposure to Low Doses of Bacterial Lipopolysaccharide and Chronic Mild Stress in Wistar Rats .

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ABSTRACT

In an attempt to link MDD with the stress biology; it was proposed that may be an 'initial common pathway' whereby immune/inflammatory and stress biomarkers combine to cause changes in brain structure and function. In the context of inflammation, pro-inflammatory cytokines (PIC) access the central nervous system and interact with the cytokine network in the brain to influence virtually every aspect of brain function relevant to behavior including neurotransmitter metabolism, neuroendocrine function, synaptic plasticity, and neurocircuits that regulate mood. Thus the present study aimed at exploring the effect of fluoxetine (FLX) a selective serotonin reuptake inhibitor (SSRI) on inflammation and depressive-like behavior induced by the combined exposure to lipopolysaccharide (LPS) and chronic mild stress (CMS) "a well-established model of depression". Animals exposed to LPS/CMS developed a depressive-like behavior that was associated with a significant increase in the serum level of corticosterone (CORT) and TNF-α levels as well as hippocampal TNF-α and the expression of nuclear factor kappa-B (NFκB). FLX treatment successfully ameliorated the depressive as well as the neuroinflammatory changes induce by LPS/CMS exposure. Thus, suggesting that the SSRI antidepressant effect may partially mediate their effectiveness via modulation of the stress-induced neuroinflammation.

Keywords: Bacterial lipopolysaccharides, chronic mild stress, hippocampus, fluoxetine, TNF-α.

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INTRODUCTION

Major depressive disorder (MDD) may be thought of as a heterogeneous syndrome rather than a single disease, to which numerous diseases of distinct causes and pathophysiologies may be contributing to its etiology leading to varying symptom constellations [1-3]. Thus, a major challenge for future research is to provide a clear stratification of MDD subtypes in order to aid diagnosis or predict treatment [4] .

Stressful life events and depression share a complex relationship, especially in individuals with certain genetic predisposition [5-7]. The onset of the first episode of depression is preceded by a severe life event in 70–80% of the cases [8]. These findings raises several questions; speculating the effect of stressors on the brain, as the proximal cause of a wide range of deleterious effects seen in different brain areas upon exposure to chronic stress and the possible molecular and neuronal effects of exposure to repeated stress.

The possibility of an 'initial common pathway' whereby immune/inflammatory and stress biomarkers combine to cause changes in brain structure and function was suggested in an attempt to link MDD with the stress biology [9, 10]. In the context of inflammation, pro-inflammatory cytokines access the central nervous system and interact with a cytokine network in the brain to influence virtually every aspect of brain function relevant to behavior including neurotransmitter metabolism, neuroendocrine function, synaptic plasticity, and neurocircuits that regulate mood [11].

Consistent with the notion that stress might provide a link between depression and inflammation, increasing data reports that stress may activate PIC, associated with depressive disorders *such as* tumor necrosis factor alpha (TNF-α), interleukin- 6 (IL-6) and their signaling pathway both in the periphery and the CNS [12]. Such findings were also observed in laboratory animals; where a variety of psychological stressors (e.g. restraint, CMS, open-field exposure or social isolation) induced an increase in the concentrations of the PICs, including IL-1b and TNF-α, in brain regions involved in emotional regulation, as well as in the periphery [13-17] .

In animals, exposure to PIC or endotoxins induces a 'sickness behavior' syndrome that is analogous to flu-like symptoms observed in human patients [18]. Sickness behavior, shares many overlapping features with symptoms of depression [19]. In animals, depressive-like behavior, reflected by decreased sucrose consumption and increased immobility (or behavioral despair) in the forced swimming test (FST) or tail-suspension test, can be induced by acute or chronic treatment with inflammatory challenges [20, 21].

Acute peripheral administration of lipopolysaccharide 'LPS' was reported to culminates in a distinct depressive-like behavioral syndrome, measured by increased duration of immobility in both the forced-swim and tail suspension tests [22]. Interestingly, chronic (but not acute) treatment with antidepressants ameliorated the LPS-induced depressive-like behavior in rats [23, 24]. With respect to the temporal aspects of symptom expression, sickness behavior develops rapidly after administration of LPS in animals and usually peaks 2–6 h post-treatment. Sickness behavior gradually resolves after 6 h and evolves into depressive-like behavior 24 h post-LPS [25, 26].

Systematic validation of the endotoxin; or cytokine-induced anhedonia as an animal model of depression is still not consistent. Yet; The striking similarities between symptoms of MDD in humans and the behavioral and physiological alterations induced by endotoxin or cytokine exposure in rodents offer a convincing 'face validity' for this model [19]. However, a recent study by El Garf et al. [16]suggested a validated animal model that addresses the possible interaction between stress and immune-inflammatory pathways in the pathogenesis of depression [16]

Thus, present study is designed to examine the behavioral and inflammatory changes induced by repeated exposure of male Wistar rats to low doses of LPS followed by exposure to CMS; a well-established model of depression. Furthermore, it is also planned to study the effects of chronic administration of a selective serotonin reuptake inhibitor; fluoxetine (FLX) on behavioral and inflammatory changes induced by repeated exposure to low doses of LPS and CMS.

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MATERIALS AND METHODS

Animals

Male Wistar albino rats weighing 200-250 g were used in the present study. Rats were allowed at least 2 weeks to acclimatize. Animals were housed individually and received pelleted rat chow (Meladco chow, El Obour, Egypt) and tap water *ad libitum* for the duration of the experiments, unless otherwise recommended by the study protocol. Temperature was maintained at 22 ± 2^{o}C and the light cycle was held at 12:12 h with lights on at 5 am. All handling were performed by the same investigator throughout the study. Animal handling and experimental protocols were approved by the Research Ethical Committee of the Faculty of Pharmacy, Cairo University (Cairo, Egypt), and comply with the Guide for the Care and Use of Laboratory Animals [27].

Treatments

LPS (Escherichia coli serotype O55:B5) and FLX were purchased from Sigma-Aldrich (St Louis, MO, USA). Drugs solutions were freshly prepared by dissolving compounds in sterile endotoxin-free isotonic. Saline LPS was administered at a dose 50 µg/kg [16] and FLX was administered at a dose of 10 mg/kg [28].

Experimental Design

Training for sucrose solution intake and groups distribution

After 2 weeks of initial habituation to the laboratory conditions, animals were trained for 48 hrs to drink 2% (w/v) sucrose (Fluka Chemical Co., Buchs, Switzerland) solution before the start of the CMS battery. Following initial measurement of sucrose preference animals were divided into 4 groups (n=10, each) with similar average SP:

I. **SAL (n=10):** control rats, received a daily i.p injection of saline (0.5ml/rat) for 6 weeks and examined by the end of the 6th week.
II. **FLX (n=10):** positive control rats received i.p injections of saline (0.5 ml/rat) every other day over 2 weeks, followed by a daily i.p injection of FLX for another four weeks.
III. **LPS/CMS (n=10):** received LPS every other day (a total of six doses) over 2 weeks, followed by exposure to CMS protocol **(Table 1)** while being injected daily with saline (0.5ml/rat) i.p for another four weeks
IV. **LPS/CMS_FLX (n=10):** received LPS every other day (a total of six doses) over 2 weeks, followed by exposure to CMS protocol (Table 1) while being treated with FLX for another four weeks.

Application of the chronic mild stress "CMS" battery

Rats were exposed to 2- 5 mild stressors per day for 4 weeks **(Table 1).** Stressors were presented in a semi random sequence over the course of a week and repeated in successive weeks, with slight shifting between the days [3]. Stressors included, water and/or food deprivation; restricted food access (i.e. 2–3 chow pellets) after food deprivation; empty water bottles after water deprivation; cage tilting; 24 h lighting; pairing; stroboscopic light and intermittent white noise (85 dB); cold temperature (≈10 °C).

Tissue and blood sampling

Rats were euthanized using i.p. urethane (1.2 g/kg). Hippocampi were dissected under strict cooling conditions and stored at -20°C until further assay. The protein concentration was determined with the Bio-Rad protein assay reagent (Bio-RAD, Richmond, USA) according to the kit instruction manual using bovine serum albumin as a standard. Blood samples were taken from the animals between (15:00-17:00) *i.e.* near the beginning of the dark cycle in order minimize the effect of circadian rhythm on the results. Serum was collected and stored at -20^{0}C.

ISSN: 0975-8585

Table 1: Schedule of stressors application during a period of one week

Day	Light cycle		Dark cycle	
	Duration	Stressor	Duration	Stressor
Sat	9 am – 5 pm	Stroboscopic light	5 pm- 9 am	Cage tilting Foreign body
Sun	9 am – 5 pm	Pairing Noise	5 pm- 9 am	Soiling the cage
		Cold restraint (30 mins)		
Mon	5 am – 5 pm	Food/ water deprivation Soiling of the cage	5:00pm - 5:30pm	Sucrose preference test
			6 pm- 9 am	Stroboscopic light
				Foreign body
Tues	9 am – 5 pm	Water deprivation	5 pm- 9 am	Cage tilting Foreign body
	9 am -11 am	Empty bottles		
Wed	9 am – 5 pm	Pairing Noise	5 pm- 9 am	Food/ water deprivation
		Cold restraint (30 mins)		
Thurs	9 am – 5 pm	Stroboscopic light Foreign body	5 pm- 9 am	Reversed light/dark cycle
	9 am -11am	Restricted food		
Fri	Continuous illumination			

Slight variation in the stressors application was adopted to avoid habituation. Saturday and Sunday light cycle stressors were shifted every other week. Tuesday and Wednesday light cycle stressors were also shifted every other week except for the empty bottle stressor it was always on Tuesday.

Measurements

Final body weight gain

Change in final weight gain was calculated as the difference between the final and baseline body weight.

Behavioral Testing

Sucrose preference test (SPT)

Anhedonia was measured by calculating the reduction in the consumption of sweet solution or preference as an indicator to the decrease in rewarding properties. SPT was scheduled at the beginning of the dark cycle [29]. After a 23 hour period of food and water deprivation, animals were presented simultaneously with two pre-weighed bottles, one bottle containing (2%) sucrose and the other water for a period of 30 minutes. The position of the 2 bottles (right/left) was varied randomly from trial to trial" during the training period" and also on the weekly test. Bottles were weighed again after the test to calculate amount consumed from each solution. Sucrose preference was calculated from the following formula:

$$Sucrose\ preference = \frac{Sucrose\ intake\ (g)}{Sucrose\ intake\ (g) + Water\ intake\ (g)} \times 100 \quad [30]$$

Forced swimming test (FST)

FST was performed according to [31] in order to confirm the ability of this stress paradigm to increase the immobility time, an indicative of depressive behavior. Animal behavior was recorded by a video camera, and immobility time was measured with a stopwatch.

Open-field test (OFT)

Open field test (OFT) was used to detect spontaneous locomotion and anxiety-related behaviors in rats. The apparatus consists of a large black arena 90 x 90 x 60 cm, divided by strips into 25 equal small squares

illuminated by white light. Parameters registered for 5 min were number of crossed squares (visited with all four feet on one square), frequency of rearing, number of entries to central zone (central four squares), latency to leave central zone [32, 33].

Biochemical Measurements

Serum corticosterone (CORT)

Serum levels of CORT α was determined using the commercially available ELISA kit "Corticosterone Rat/Mouse ELISA" (DRG international®, USA), according to the manufacturer's instructions. Absorbance was measured at 450 nm and the lowest analytical detectable level of CORT was 4.1 ng/ml.

Serum TNF- α

Serum levels of TNF-α was determined using the commercially available ELISA kit "Rat TNF-α Platinum ELISA" (eBioscience, CA, USA) according to the manufacturer's instructions. Absorbance was measured at 450 nm and the lowest analytical detectable level was 11 pg/ml.

Hippocampal expression of NFκB mRNA by quantitative RT-PCR

Total RNA was extracted from hippocampal tissue homogenates using RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Reverse transcription into cDNA was performed from the total isolated RNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA) following the manufacturer's instructions. Rt-PCR reaction was performed using SYBR green PCR Master Mix (Applied Biosystems, CA, USA). PCR reactions were set up in a total volume of 50 µl; containing 25 µl SYBR Green Mix (2x), 0.5 µl cDNA, 2 µl primer pair mix (5 pmol/ µl each primer) and 22.5 µl H2O. The forward and reverse primers [34] were checked for correctness of the gene order according to the GenBank **(Table 2).** The relative expression of the studied genes was calculated after normalization to the internal standard β-Actin [35]. All reactions were run on a StepOne™ Plus RealTime PCR system (Applied Biosystems) using the default settings recommended by the manufacturer, and analyzed using StepOne software v2.3.

Table 2:

Gene	Gene Accession no.	Primer		Reference
NFκB	NM_199267	Forward Backward	**5**-GCG CAT CCA GAC CAA CAA TAA C-**3** **5**-GCC GAA GCT GCA TGG ACA CT-**3**	[33]
β-Actin	NM_031144	Forward Backward	**5**-GCC ATG TAC GTA GCC ATC CA-**3** **5**-GAA CCG CTC ATT GCC GAT AG-**3**	[34]

Hippocampal TNF-α

The hippocampal TNF-α level were determined using the commercially available ELISA kit, Rat TNF-alpha ELISA Kit (ELR/TNFalpha-001C - RayBiotech, Georgia, United States). Absorbance was measured at 450 nm and the lowest analytical detectable level was 25 pg/ml.

Statistical Analyses

The statistical analyses of the Data were analyzed and graphically presented using the statistical software package ***"Graphpad Prism, version 5"*** (GraphPad software Inc., CA, USA. Normality was assessed by D'Agostino-Pearson normality test. Parametric data were expressed as mean ± SEM and statistical comparisons were carried out using one-way ANOVA followed by Tukey Multiple Comparisons Test. Nonparametric data, data were expressed as median/interquartile range and Kruskal–Wallis (KW) test was utilized followed by post-hoc Dunn's test. The minimal level of significance was identified at $p < 0.05$.

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RESULTS

Final body weight gain

Notably in the positive control group; the classical antidepressant FLX induced a remarkable decrease in the final body weight gain by 71.37% in comparison to the SAL control group. The combined LPS/CMS exposure induced a 30.31 fold decrease in the final body weight gain in comparison to the SAL group. However, rats treated with FLX a classical antidepressant known to induce weight loss, showed a further 16.08 fold reduction in the final body weight gain compared to the LPS/CMS **(fig. 1A)**. Noteworthy, the LPS/CMS - FLX group showed a drastic reduction in the body weight gain even in comparison to the FLX positive control group by 1.85 fold. The differences among the studied groups were statistically significant as calculated by One-way ANOVA [$F_{(3, 36)} = 47.05$, $p < 0.0001$].

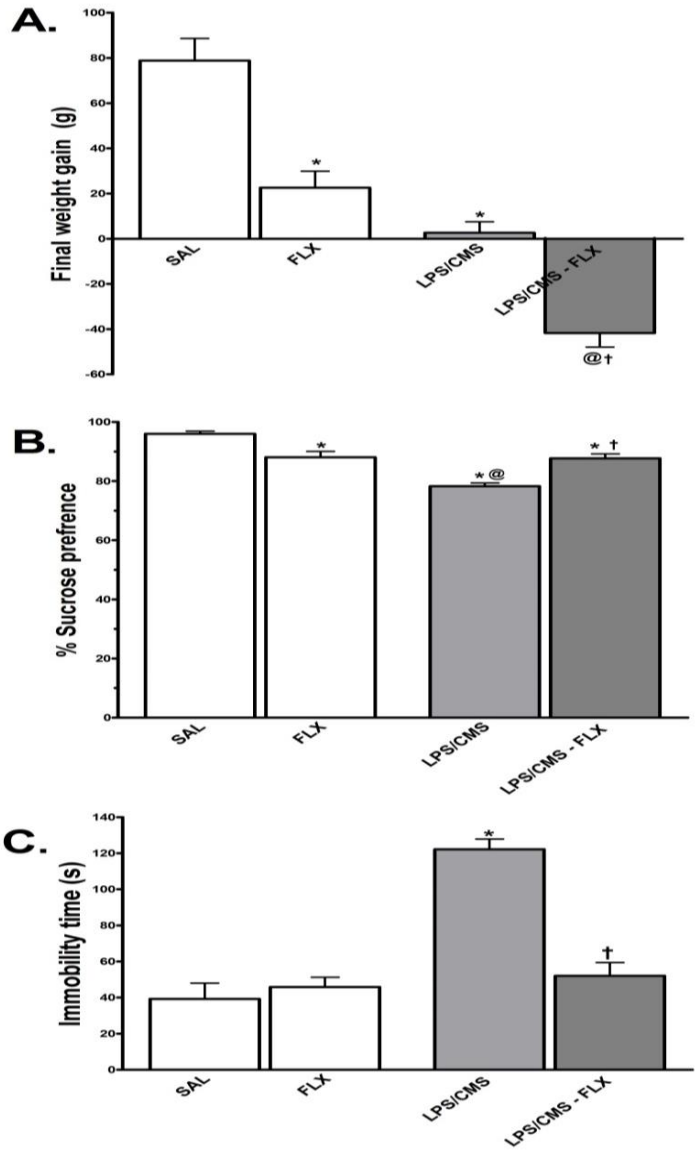

Figure 1: Effects of LPS/CMS exposures and FLX treatment on weight gain and behavioral changes

Bar graphs illustrating changes in: **(A)** Final weight gain; **(B)** SPT and **(C)** FST among groups. Data are presented as mean ± S.E.M. SAL (n=10). For comparisons among groups, one-way ANOVA followed by the post hoc "Tukey's Multiple Comparison Test" was utilized. ***p*<0.05 significant in comparison to SAL, [@] *p*<0.05 significant in comparison to FLX and [†] *p*<0.05 significant in comparison to LPS/CMS.

SPT: Sucrose preference test; **FST:** Forced swim test; **SAL:** Saline**; LPS:** Lipopolysaccharides; **CMS:** Chronic mild stress.

ISSN: 0975-8585

Behavioral testing

SPT

LPS/CMS exposure induced a remarkable reduction in the SP by 18.45% in comparison to the control. Notably, the positive control group treated with FLX showed a significant decrease in the SP by 6.37% compared to the control group. Hence; the effect of FLX treatment on the LPS/CMS induced changes in SP would be compared to the positive control rather than the control group receiving saline. Indeed, LPS/CMS-FLX showed a significant improvement in the SP that was comparable to that of the FLX positive control group and lower than LPS/CMS group by 12.01% **(fig.1B).** Interestingly, the SP scored by the LPS/CMS rats was similar to that scored by the FLX positive control group. The differences among the studied groups were statistically significant as calculated by One-way ANOVA [$F_{(3, 36)} = 26.63$, $p < 0.0001$].

FST

LPS/CMS exposure engendered a significant increase in the desperate behavior by 3.12 fold in comparison to the control group. Noteworthy, FLX positive control group did not show any alterations in the despair behavior compared to control rats. This escalation in the d*e*sperate behavior was significantly amended in by FLX treatment in LPS/CMS – FLX group; which demonstrated a 57.36% reduction in the immobility time in comparison to LPS/CMS exposed rats. Remarkably, immobility time scored in the LPS/CMS – FLX group was comparable to that scored by the control group **(Fig. 1C).** The differences among the studied groups were statistically significant as calculated by One-way ANOVA [$F_{(3, 36)} = 30.74$, $p < 0.0001$].

OFT

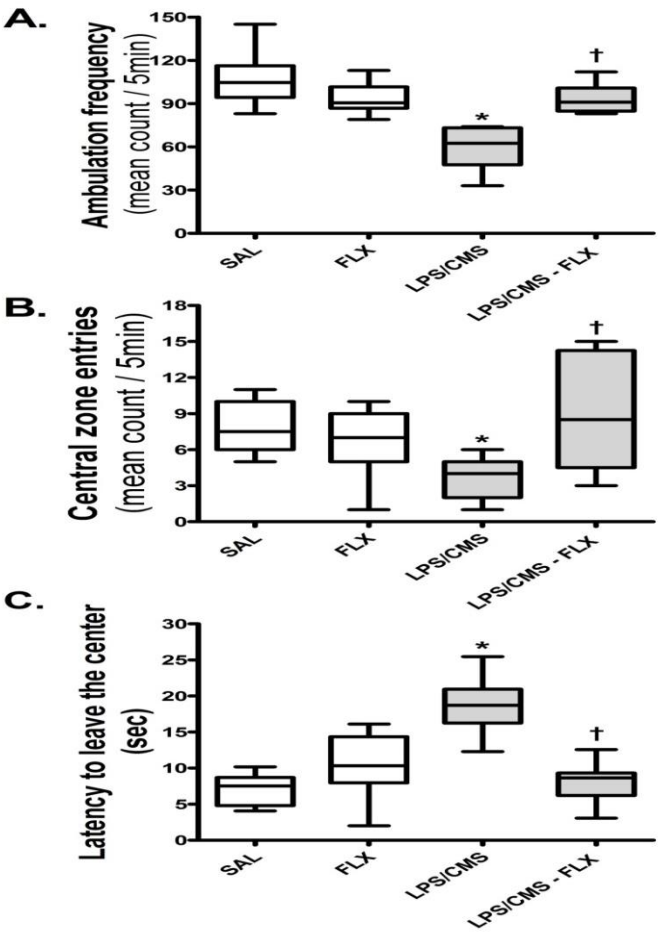

Figure 2: Effects of LPS/CMS exposures and FLX treatment on the behavioral changes in open field test.

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Box-Whisker graph illustrating changes in the OFT in rats among the different groups; (A) Central zone entries; (B) Total ambulation; (C) Latency to leave the center zone. (D. Data are presented as median/ interquartile range. (n=10). For comparisons among groups, Kruskal-Wallis Test followed by post hoc "Dunn's Multiple Comparisons Test" was utilized. *p<0.05 significant in comparison to SAL, † p<0.05 significant in comparison to the LPS/CMS.
CORT: Corticosterone; **TNF-α:** Tumor necrosis factor alpha; **SAL:** Saline**; LPS:** Lipopolysaccharides; **CMS:** Chronic mild stress.

Statistical analysis revealed that exposure LPS/CMS exposure instigated a significant reduction in the total ambulation frequency by 40.19% [KWS=24.69, p < 0.0001] and the frequency of central zone entries [KWS=13.15, p=0.0043]. Furthermore, the combined model induced a significant increase in the latency time to leave the central zone [KWS=24.1, p < 0.0001]. Treatment with FLX successfully ameliorated all of the aforementioned parameters (fig.2). Noteworthy, FLX positive control group did not show any alterations in the behavioral changes assessed in the OFT compared to control SAL rats.

Biochemical measurements

Serum CORT

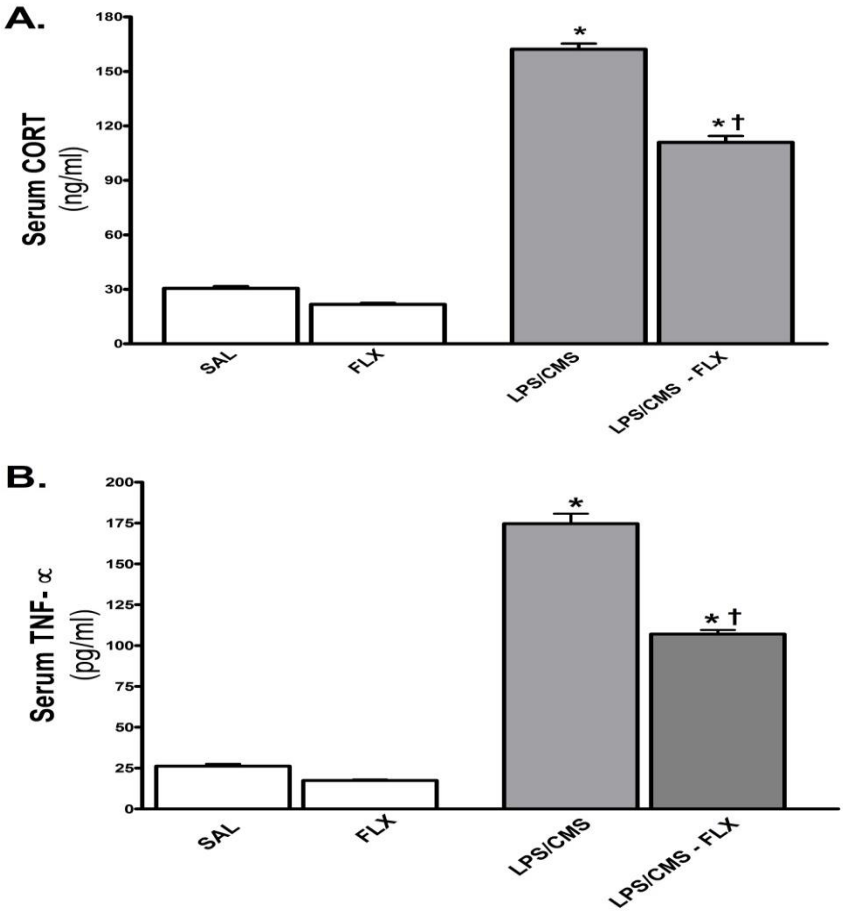

Figure 3: Effects of LPS/CMS exposures and FLX treatment on serum biomarkers

Bar graph illustrating changes in the serum level of **(A) CORT and (B) TNF-α** among groups. Data are presented as mean ± S.E.M (n = 6). For comparisons among groups, one-way ANOVA followed by the post hoc "Tukey's Multiple Comparison Test" was utilized. *p<0.05 in comparison to SAL, and † p<0.001 significant in comparison to LPS/CMS.
CORT: Corticosterone; **TNF-α:** Tumor necrosis factor alpha; **SAL:** Saline**; LPS:** Lipopolysaccharides; **CMS:** Chronic mild stress.

A significant difference in the serum CORT was observed among the different groups as calculated by one-way ANOVA [$F_{(3, 20)}$ = 749, p < 0.0001]. The combined LPS/CMS exposure induced a 5.3 fold in comparison to the SAL group. However, rats treated with FLX demonstrated a significant reduction in the serum level of TNF-α by 31.57% in comparison to LPS/CMS group **(fig.3A).**

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Serum TNF-α

A significant difference in the serum level of TNF-α was observed among the different groups as calculated by one-way ANOVA [$F_{(3, 20)} = 483.4$, $p < 0.0001$]. The combined LPS/CMS exposure induced a 6.66 fold in comparison to the SAL group. However, rats treated with FLX demonstrated a significant reduction in the serum level of TNF-α by 38.72% in comparison to LPS/CMS group **(fig.3B).**

Hippocampal NFκB

A significant difference in the expression of NFκB was observed among the different groups as calculated by one-way ANOVA [$F_{(3, 20)} = 38.13$, $p < 0.0001$]. LPS/ CMS exposure prompted a significant increase in the expression of NFκB by 8.46 folds in comparison to the control group. However, FLX treatment significantly decreased the LPS/CMS induced enhancement in the expression of NFκB by 34.31% in comparison to LPS/CMS-FLX group**.** Notably, FLX positive control group did not show any alterations in the expression of NFκB mRNA **(fig.4A).**

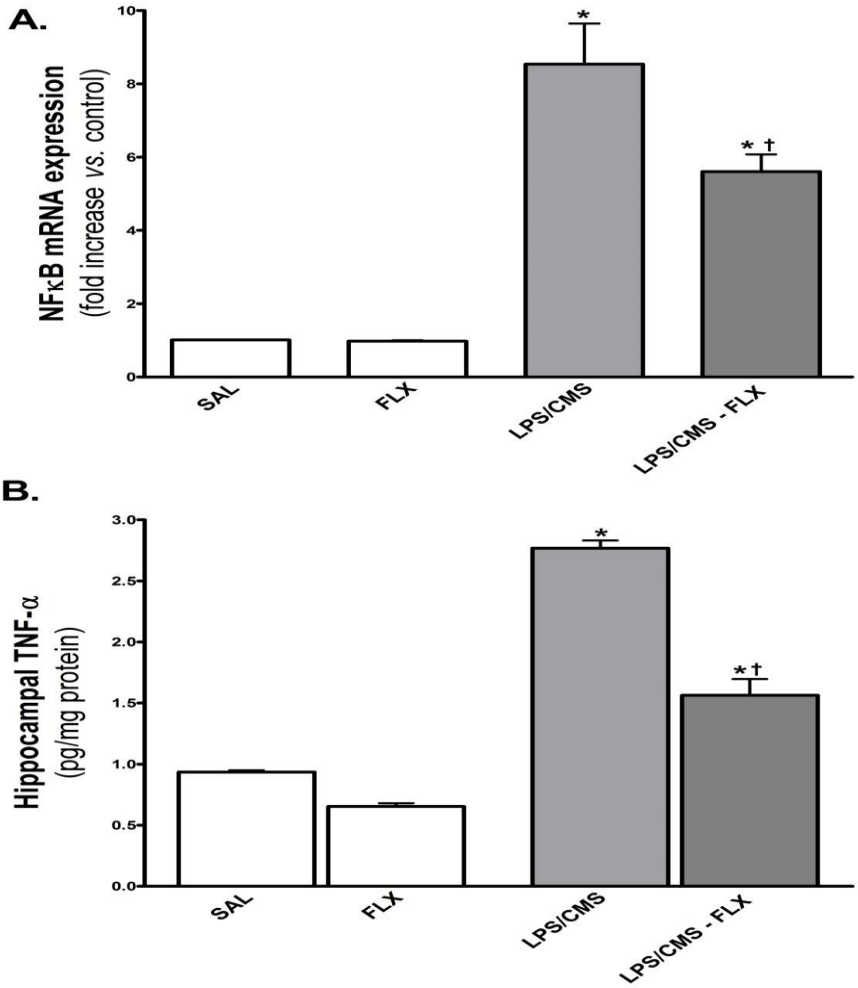

Figure 4: Effects of LPS/CMS exposures and FLX treatment on the hippocampal expression of NFκB and TNF-α level

Bar graph illustrating changes in the relative expression of the hippocampal **(A)** NFκB mRNA **and (B)** TNF-α level. Data are presented as mean ± S.E.M (n = 6). For comparisons among groups, one-way ANOVA followed by the post hoc "Tukey's Multiple Comparison Test" was utilized. *p<0.05 in comparison to SAL, and † p<0.001 significant in comparison to LPS/CMS.

NFκB: Nuclear factor kappa B; **TNF-α:** Tumor necrosis factor alpha; **SAL:** Saline**; LPS:** Lipopolysaccharides; **CMS:** Chronic mild stress.

Hippocampal TNF-α

A significant difference in the hippocampal level of TNF-α was observed among the different groups as calculated by one-way ANOVA [$F_{(3, 20)} = 157$, $p < 0.0001$]. The combined LPS/CMS exposure instigated a profound elevation in the hippocampal level TNF-α by 2.95 fold compared to the control group. Conversely, FLX treatment mitigated such an enhancement by 43.68% **(fig.4B).** Remarkably**,** FLX positive control group did not show any alterations in the hippocampal level of TNF-α.

DISCUSSION

In the present study, we report that chronic administration of FLX attenuated the enhanced combined role of immune activation and stress on the development of depressive-like behavior and neuroinflammation in male Wister rats' exposed to the combined exposure LPS/CMS.

Interestingly; in the presented study the utilized combined LPS/CMS model presents a new animal model of depression that may addresses depression as a multifactorial disease resulting from the interaction of several environmental insults (*e.g*. stressors) and genetic predisposition, which would ultimately give rise to the depressive disease state. Precisely, this combined model address the similarity between.

Each stressful exposure; either the CMS alone [15, 36] or LPS alone [19, 24, 37, 38] was previously documented by several investigators to engender both depressive behavior as well as cytokine imbalance in rodents.

However a recent report from our lab was the first to enlighten that the combined exposure to peripheral immune challenge (LPS) followed by chronic mild stress could instigate an analogous or even a more compelling depressive and neurochemical profile than each exposure alone [16]. A further validation of this suggested model is provided by our present study; where the combined LPS/CMS exposure induced a notable despair behavior and anhedonia as measured by immobility time in FST [39] test as well as the percentage of sucrose consumption in SPT [40]. Effectively, FLX treatment significantly improved these behavioral alterations, where it induced a significant reduction in the despair behavior scored in the FST and a notable enhancement in the SP compared to the LPS/CMS group. Such improvements are a typical characteristic feature for clinically effective antidepressant drugs in ameliorating the behavioral effects of both exposures "CMS" [3, 15] and "LPS" [16, 23, 24].

A further addition to the aforementioned depressive profile was furnished by the alterations observed in the OFT. LPS/CMS exposure induced a significant decline in the total ambulation frequency and the central zone entries as well as a notable increase in the latency time to leave the central zone. Such findings are in accordance with previous reports underscoring the association of chronic stress exposure with the decrease in locomotor activity and enhanced anxiety [40-42]. FLX treatment significantly ameliorated these changes, as previously reported by other studies [23, 43].

Furthermore; these depressive behaviors induced by the combined LPS/CMS exposure were accompanied by significant reduction in body weight gain; an important diagnostic criteria of the major depressive disorder as indicated by (DSM- IV-TR). This finding is in line with those of other investigators who reported that chronic stress exposure [3, 30, 44-46] as well as LPS [16, 23, 47] induced such an effect. Thus adding a further validation of our combined LPS/CMS model as an effective depressive model and a supplementary physiological index for the incidence of stress [46, 48]. As expected, this effect was not prevented by FLX treatment as formerly declared in the literature [49]. However; FLX treatment induced a significant weight loss in both control and stressed rats. This pardox may be attributed to the fact that enhancement of the serotonergic system, alters food intake both quantitatively and qualitatively and decrease the leptin level even in the absence of stress exposure [43, 49].

The aforementioned LPS/CMS induced depressive-like behaviors were escorted by a significant increase in the serum level of CORT and TNF-α. Furthermore, this imbalanced cytokine profile was reflected centrally, as exhibited by the enhanced expression of the hippocampal NFκB and its downstream player TNf- α.

Noteworthy, peripheral and central inflammation operates in parallel, where the brain is no longer considered as an immune privileged organ as a bidirectional communication seems to link the central and

peripheral inflammation [50, 51]. Numerous attempts were proposed to elucidate the cytokine-induced depression in the literature [50, 52, 53], however; the exact mechanism remains poorly understood due to the complexity and diversity of the processes involved [51].

Several lines of evidence support the implication of cytokines in modulation of the HPA-axis function [11, 54] as well as serotonergic neurotransmission [16, 55]. Our findings are just in line with previous reports associating the exposure of rats to "CMS and/or LPS" with the simultaneous increase in the serum level of CORT and TNf- α [16, 56]. One mechanism by which chronic cytokine exposure may influence the HPA-axis is through the inhibitory effects of inflammatory signaling molecules such as NFκB on the glucocorticoid receptors [57].

The LPS/CMS induced observed depressive behavior could be also attributed to the TNF-α induced activation of tryptophan-IDO pathway [15, 16]. Peripheral LPS injections induced depressive-like behaviors through the pro-inflammatory cytokine induced, indoleamine-2, 3-dioxigenase (IDO) enzyme [50, 55, 58, 59], and SERT activation in the brain [60].Therefore, peripheral and central inflammation would be involved in depressive-like behavior in mice treated with LPS [37]. Thus, highlighting the perception that peripheral inflammation may relay signals to the central nervous system [50, 53]. Interestingly, FLX treatment ameliorated the LPS/CMS induced elevation in serum CORT and TNF-α.with a down regulation in the expression of hippocampal NFκB and its downstream hippocampal TNF-α. The above data support the concept that the anti-inflammatory effects of SSRI

The findings of the presented study, reinforce the notion that the PIC "TNF-α" may play an important role in the pathophysiology of depression via its potential modulatory effect on the HPA-axis and the serotonergic neurotransmission. Thus targeting the PIC may augment the antidepressant effect of traditional therapies. Furthermore, it's likely to assume that the anti-inflammatory effect of the traditional antidepressants FLX [37, 61] may play an important role in alleviating the depressive system especially in the subtype of depressive patients that demonstrate systemic inflammation by inhibiting peripheral and central inflammation.

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