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Original Article

Low Dose Lipopolysaccharide-Induced Depressive-Like Phenotype is Mediated by Proinflammatory Cytokines in Mice and Role of Ketamine

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ABSTRACT

Background: Depression is a common mental illness, with an estimated 3.8% of global population affected. In the pathophysiology of depression, ketamine acts quickly in patients. Treatment with low-dose ketamine upon administration to stressed C57BL/6J mice is now a major translational research area to facilitate further innovation. **Objectives:** The present work was aimed to establish a depressant like animal model after 6 days of LPS injection, where LPS did not promote body weight loss. **Materials and Methods:** Peripheral administration of low dose of Lipopolysaccharide (LPS) activates cytokines and culminate in a distinct depressive-like behavioral syndrome, measured by increased duration of immobility in the forced swim and anhedonia in sucrose preference tests. Cytokines like TNF-α, IL-6, IL-1β and IFN-γ were determined in brain homogenate and in plasma using western blot performed with automated Jess system (ProteinSimple) and ELISA respectively. **Results:** Ketamine prevents development of depressive-like behavior by decreasing swimming behavior and increasing preference to sucrose in stressed animals. Ketamine treatment reduced the LPS induced secretion of IFN-γ (p<0.05 for 30 mpk), IL-6 (p<0.05 for 30 mpk), TNF-α (p<0.0001 for 30 mpk) and IL-1β in plasma. Similarly, ketamine treatment reduced the LPS induced secretion of IFN-γ (p<0.001 for 10 and 30 mpk), IL-6, TNF-α (p<0.01 for 10 and 30 mpk) and IL-1β (p<0.05 for 10 mpk, p<0.0001 for 30 mpk) in brain. The plasma and brain concentrations of ketamine were analysed using LC-MS/MS and Brain/Plasma ratio (B/P) of ketamine at 10 and 30 mpk were calculated as 0.70 and 0.82 respectively. **Conclusion:** In summary, these data emphasizes that ketamine treatment modulate cytokine level, showed good brain to plasma exposure and provides its anti-stress effects in the C57BL/6 mouse strain, which may be possible reason for the anti-depression property and is relevant to human stress-induced depression.

Keywords: B/P, Cytokines, Depression, LPS, FST, SPT.

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INTRODUCTION

The World Health Organization (WHO) estimates that more than 350 million people which is around 3.8% of global population[1] are now living with depression. LPS, which triggers the Toll-like receptor 4 transmembrane cytokine pathway, acutely activates the peripheral innate immune system in experimental mice.[2] Increased immobility in the forced swim test and tail suspension test, a reduction in sweetened solution consumption and a suppression of sexual behaviour are all signs that LPS causes depressive-like behavior.[3]

LPS causing inflammation may enhance the delivery of both small and large molecules to the brain, improving the accuracy of treatments or diagnostic procedures. This strategy must be balanced between the dangers of systemic inflammation and severe animal weight loss.[4]

Moreover, depression considered as a stress-linked disease with significant morbidity and the anesthetic drug Ketamine (Ket) is of growing interest in the treatment of depression. In rodents, chronic unpredictable mild stress, chronic social defeat stress and lipopolysaccharide induced depression are more sensitive and appropriate than normal mouse Forced Swim Test (FST).[5]

Ket is a non-competitive NMDA receptor antagonist, which is a class of phencyclidine (N-1-phenycyclohexypiperidine, PCP) binds to the phencyclidine site to produce anesthesia.[6]

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Earlier investigation showed that Ket exerted an anti-inflammatory effect in the surgery of sepsis patients.[7] It can rapidly relieve depressive symptoms and suicidal ideation in many patients[8] and promotes sustained antidepressant effects in the chronic mild stress and social defeat stress models.[9] Intranasal administration of esketamine developed by Janssen Pharmaceutical reported to have antidepressant actions.[10]

Ket at the doses 3 mpk and 1 mpk significantly decreased the immobility duration in the FST and TST respectively; however it was non-effective at lower doses[11] and at 90-100 mpk, intraperitoneal injection is lethal and used to euthanize the mice.[12]

The rapid-acting antidepressant ketamine has an impact on mice behaviour and reactions based on the gender of the human researchers. They further demonstrated that exposure to male fragrance activates Corticotropin-Releasing Factor (CRF) neurons projecting from the entorhinal cortex to the hippocampus prior to ketamine administration and that CRF is essential and sufficient for ketamine's *in vivo* and *in vitro* effects.[13]

Therefore, we male experimenter attempted to induce a depression-like phenotype in mice by giving them LPS over a period of six days without observable changes in body weight and assessed the sub anaesthetic dose of Ket.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice (20 to 25 g) were procured from a single source (Vivobiotech, Hyderabad) and housed in a group of 4 in individually ventilated cages. Animals were given an autoclaved SDS pelleted feed and autoclaved water *ad libitum*. Mice were kept with temperature 21-24°C and relative humidity maintained at 40-70%. With registration number IAEC/JDC/2018/150, the Institutional Animal Ethics Committee of Jubilant Biosys Ltd, Bengaluru accepted the animal protocol.

Reagents

Lipopolysaccharide (LPS, *Escherichia coli, serotype* O111:B4, L2630, Lot# 028M4022V, Sigma), Ketamine (Lot # B5B0394, Baxter), RIPA buffer (9806, Cell Signaling Technology), Protease Inhibitor Cocktail (P8340, Sigma), Amitriptyline (Lot # 078K1868, Sigma), Sucrose (Lot # 57-50-1, Fisher Scientific). TNF-α, IFN-γ, IL-1β and IL-6 ELISA kits were purchased from R and D Systems Inc. Reagents for immunoblotting using Jess System (ProteinSimple) were Standard Pack reagents (Ref No.: PS-ST01-8) and antibodies for TNF-α, IFN-γ, IL-1β and IL-6 were used from CST. All other reagents were used from Sigma Chemical Co.

Experimental Groups

The group allocation and schematic representation of experimental plan were mentioned in Table 1 and Figure 1 respectively. Half of the animals from each group underwent behavioral tests (SPT, FST, Rotarod) and the other half used for biomarker analysis (Simple Wes and exposure). The mice were administered multiple i.p. injection of Ket or equal volume of physiological saline to corresponding groups for consecutive 6 days at 1 hr post LPS injection.

All behavioral experiments were performed on Day 7, 1 hr after dosing of saline or Ket to respective groups. The observer was blind to the experimental conditions.

Behavioral experiments

Mouse Sucrose Preference Test

The procedure started with an adaptation period on Day 5, where mice were exposed to sucrose solution with two bottles of 1% (w/v) sucrose solution placed in each cage. On Day 6, sucrose solution in one bottle was replaced with normal autoclaved water during 24 hr. Mice were deprived of water and food for further 6 hr, post this adaptation period. The test was performed on Day 7 after last saline or Ket injection to evaluate anhedonia. Mice were given a free choice between two bottles containing 100 mL of 1% w/v sucrose solution and 100 mL of autoclaved water for the sucrose preference test. The bottles were weighed at the start and 1 hr during the trial and the amounts of water and sucrose solution consumed were noted. The % sucrose preference was reported.

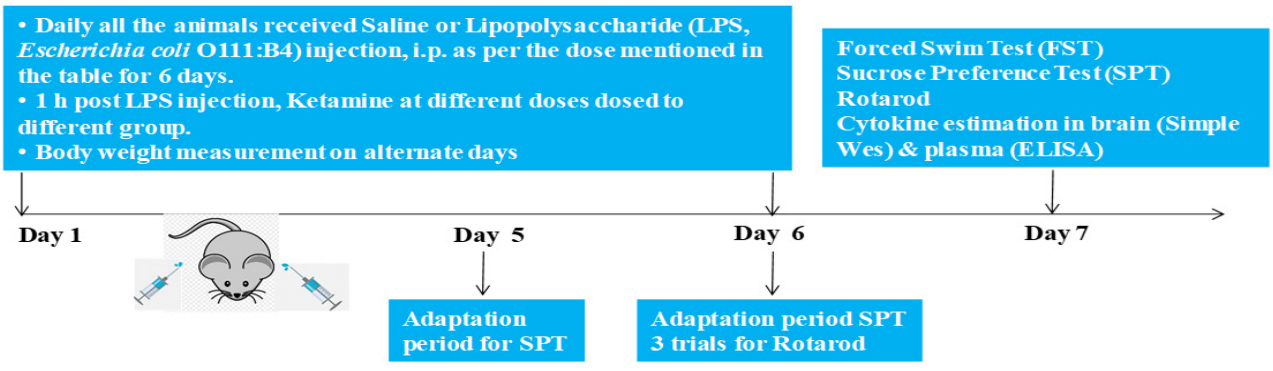


Figure 1: Schematic representation of experimental plan.

Table 1: Group allocation.

Groups	Dose	Route of administration	n
Normal control	0.9% saline.	i.p.	8
Disease control	LPS @ 300 µg/kg.	i.p.	8
Treatment low dose	Ket @3 mpk+LPS @ 300 µg/kg.	i.p.	8
Treatment middle dose	Ket @10 mpk+LPS @ 300 µg/kg.	i.p.	8
Treatment high dose	Ket @30 mpk+LPS @ 300 µg/kg.	i.p.	8

Mouse Forced Swim Test

Briefly stated, each mouse was lowered into a glass cylinder with a diameter of 23 cm and a height of 30 cm that contained 14 cm of water that was kept at a temperature of 24±1°C. Between each testing session, the water was changed. The mice were kept in the cylinder for 6 min before being put back in their cage. Over the final 5 min of the test, the immobility time was calculated. When mice floated passively in the water, moving only enough to keep their heads above the water's surface that condition was referred to as immobility.[5]

Motor Coordination using Rotarod Test

The motor was set to acceleration mode which started from a low speed and accelerates within 1 min to a preset maximum speed of 20 rpm with a cut off time 180 sec. On Day 6, the animals were placed on the rotarod (47600-Mouse Rota-Rod, UGO BASILE) and three trials were given for each animal. The main experiment was performed on Day 7. Test was carried at 30, 90 and 150 min time points post treatment and average longest time spent on rotarod was observed.[14]

Tissues collected at the time of necropsy for analysis

On Day 7, after the experimental regimen (1 hr following saline/Ket injection), half of the mice were slightly anesthetized under CO_2 asphyxia, blood collected from the retro-orbital sinus of mice in an eppendorf tube containing Na-EDTA as an anticoagulant. Centrifugation was used to extract the plasma (5000 g, 4°C, 5 min), which was then stored at -80°C until analysis. The whole brain was extracted from the animal after it had died from CO_2 asphyxia and it was promptly snap frozen in liquid nitrogen and kept at -80°C for exposure analysis. The remaining set of animals after performing SPT, FST and Rotarod were sacrificed by cervical dislocation and whole brain was removed, immediately snap frozen in liquid nitrogen and stored at -80°C till cytokine analysis.[15]

Immunoblotting

The quantity of TNF-α, IFN-γ, IL-1β and IL-6 were determined by Western-blot using the Jess fully automated system (ProteinSimple; Bio-Techne) following the recommended protocol. The band area was calculated using Compass (V 3.1) software. The ratio of cytokine to housing keeping protein (GAPDH) was analyzed and the fold over to disease control, % inhibition of disease by treatment groups was calculated and represented in the graph.[16]

Plasma and brain processing method for Ket in LC-MS/MS Assay by protein precipitation method

10 µL of the IS working stock solutions were added to an aliquot of 50 µL of plasma and brain homogenate and vortexed for 10 sec. Then, 300 µL of acetonitrile was added, vortexed for 2 min and centrifuged for 5 min at 14000 rpm in a refrigerated centrifuge (Eppendorf 5424R) with a temperature of 5°C. A 200 µL clear supernatant organic layer was isolated after centrifugation and 5 µL of this layer was put onto the column for LC-MS/MS analysis.[17]

Statistical Analysis

Data were reported as mean±SEM of 4-8 animals. Statistical analysis was performed using Graph pad prism (version 9.0.0 GraphPad Software, San Diego, CA, USA). Comparisons of all study parameters were made between the treatment groups and respective vehicle control groups by One-way Analysis of Variance (ANOVA) followed by Dunnett's *post hoc* test. For rotarod test and % change in B/W, two-way ANOVA followed by Bonferroni post-test were used. A p value less than 0.05 were considered significant.

RESULTS

% change in B/W

There was a significant drop of B/W in LPS group on Day 3 and 5 due to LPS injection, which slowly recovered on Day 7. Ket at 3 mpk showed significant improvement in B/W on Day 3 and 5 (Figure 2, Table 2).

On day 3, when compared to Saline group, the LPS group's % body weight (-12.3±1.6 Vs 1.0±0.6) changed significantly ($p<0.0001$). Ket treatment with 3 mpk significantly ($p<0.001$) protected the body weight reduction (-12.3±1.6 Vs -4.4±2.3). Ket treatment (10 mpk) showed non-significant but meaningful improvement in body weight reduction (-12.3±1.6 Vs -8.1±0.6), whereas Ket treatment (30 mpk) did not show improvement in body weight reduction (-12.3±1.6 Vs -11.4±0.6) caused due to LPS.

On day 5, when compared to the Saline group, the LPS group's % body weight (-7.6±2.2 Vs -1.4±1.0) changed significantly ($p<0.01$). Ket treatment with 3 mpk significantly ($p<0.0001$)

protected the body weight reduction (-7.6±2.2 Vs 0.8±0.8). Ket treatment at 10 mpk showed minimal improvement and 30 mpk did not show improvement of body weight reduction (-7.6±2.2 Vs -4.5±0.4) and (-7.6±2.2 Vs -5.7±0.6) respectively caused due to LPS.

Effect of Ket on LPS induced SPT

The depressive like behavior, which assesses anhedonia, confirmed by the decrease in preference of sucrose solution to water. In mice after multiple LPS injection, there was a significant (p<0.05) decrease of preference for sucrose. The saline injected animals demonstrated ~72% preference for sucrose (1%) solution over water (Figure 3A, Table 3).

Ket treatment at 30 mpk improved the preference to sucrose as compared with the LPS group (65.6±1.1 Vs 34.9±7.2).

Effect of Ket on LPS induced FST

Mice after multiple LPS injection developed significantly (p<0.0001) increased time of immobility duration in LPS group

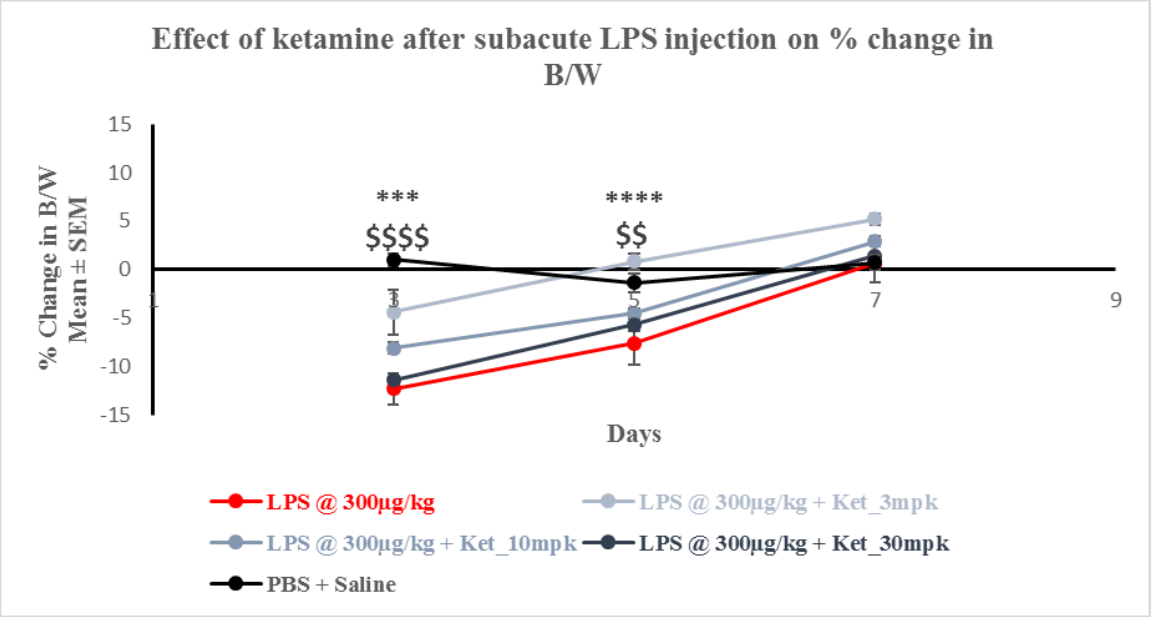


Figure 2: % Change in B/W.

Table 2: Effect of Ket on % change in Body weight after sub-acute LPS injection.

Groups	Day 3 Avg±SEM	Day 5 Avg±SEM	Day 7 Avg±SEM
PBS+Saline	1.0±0.6	-1.4±1.0	0.7±0.8
LPS @ 300 µg/kg+Saline	-12.3±1.6	-7.6±2.2 $^{\$\$}$	0.6±1.9
LPS @ 300 µg/kg+Ket @ 3 mpk.	-4.4±2.3 ***	0.8±0.8 ****	5.2±0.6
LPS @ 300 µg/kg+Ket @ 10 mpk.	-8.1±0.6	-4.5±0.4	2.9±0.5
LPS @ 300 µg/kg+Ket @ 30 mpk.	-11.4±0.6	-5.7±0.6	1.4±1.5

$^{\$\$}$ p<0.01 and $^{\$\$\$}$$p$<0.0001 vs. Saline; ***$p$<0.001 and ****$p$<0.0001 vs LPS. Two-way ANOVA followed byBonferroni post hoc test.

Table 3: Effect of Ket in LPS induced Immobility in FST and Sucrose preference in mice.

Groups	Immobility duration (sec) Avg±SEM	% Sucrose preference Avg±SEM
PBS+Saline.	74±5.1	72.3±10.3
LPS @ 300 µg/kg+Saline.	148.4±2.1	34.9±7.2$^{\$}$
LPS @ 300 µg/kg+Ket @ 3 mpk.	134.6±7	52.3±2.3
LPS @ 300 µg/kg+Ket @ 10 mpk.	131±2.5 *	50.6±3.9
LPS @ 300 µg/kg+Ket @ 30 mpk.	85.5±2.4 ****	65.6±1.1*

$^{\$}$ p<0.05 and p<0.0001 vs. Saline; *p<0.05 and ****p<0.0001 vs LPS. One-way ANOVA followed by Dunnett's post hoc test.

as compared with the control group (148.4±2.1 Vs 74±5.1, Figure 3B, Table 3). Ket treatment at 10 mpk prevented the increase in immobility time as compared with the LPS group (148.4±2.1 Vs 131±2.5) and Ket treatment at 30 mpk prevented the increase in immobility duration as compared with the LPS group (148.4±2.1 Vs 85.5±2.4).

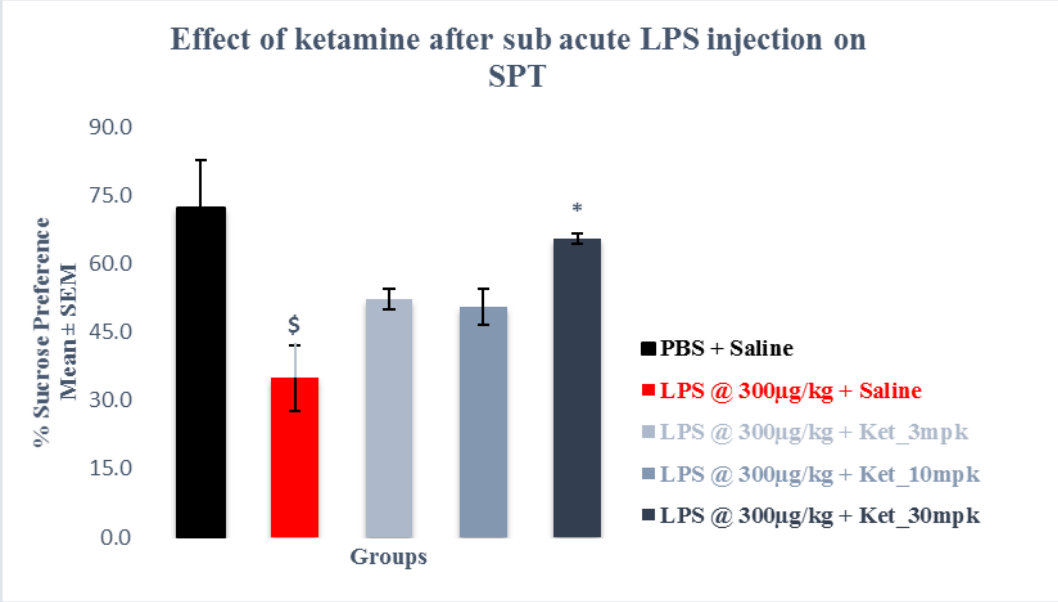
Effect of Ket on motor coordination

In the mouse rotarod test, the impact of Ket and amitriptyline on motor coordination was assessed at 30 min, 90 min and 150 min on Day 7 (Figure 4, Table 4). During the investigation, it was observed that amitriptyline at 10 mpk dose produced motor deficits as a side effect in the rotarod test at 30 min, 90 min

Table 4: Effect of Ket in LPS induced Motor coordination in rotarod.

Dose (mpk)	30 min Avg±SEM	90 min Avg±SEM	150 min Avg±SEM
Amitriptyline_10	36.1±6.6 ****	63.2±7.1****	141.7±17.6**
Ket_30	180±0	180±0	180±0

**$p<0.01$ and ****$p<0.0001$ vs. LPS+Saline, Two Way ANOVA, with post hoc Bonferroni tests.



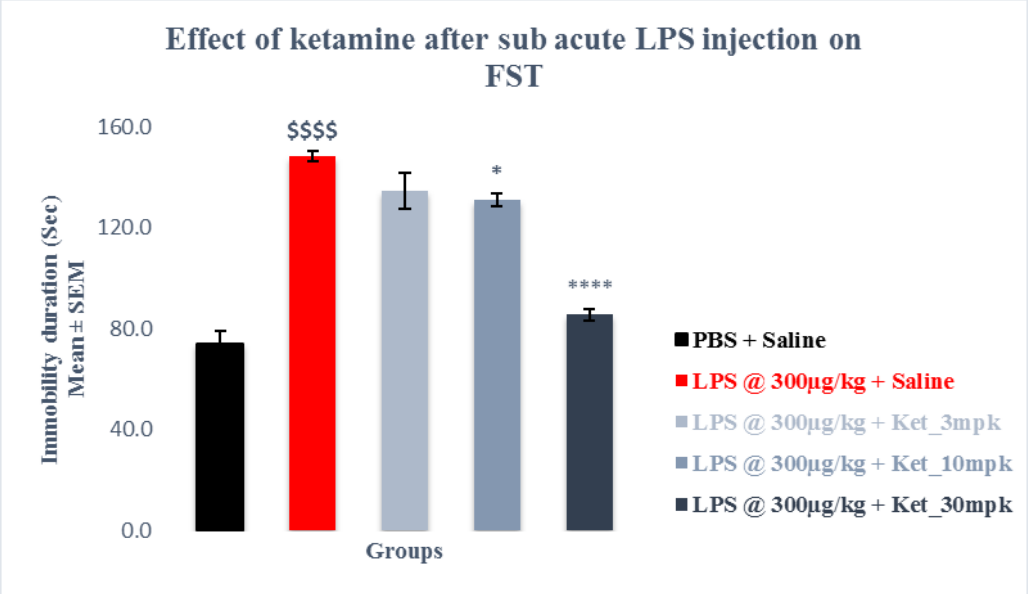


Figure 3: % SPT [A], FST [B]

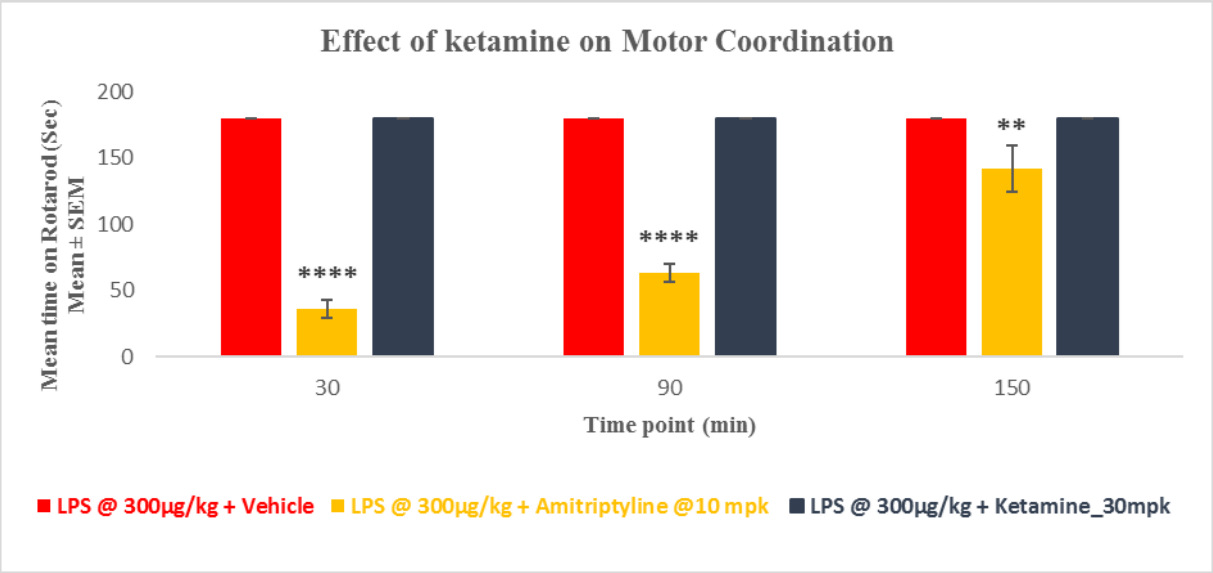
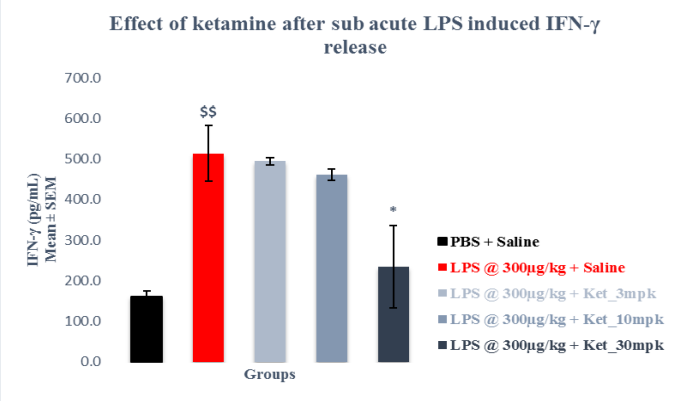
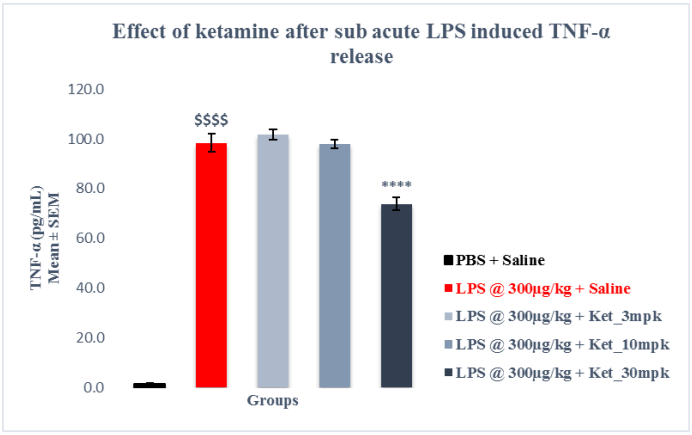
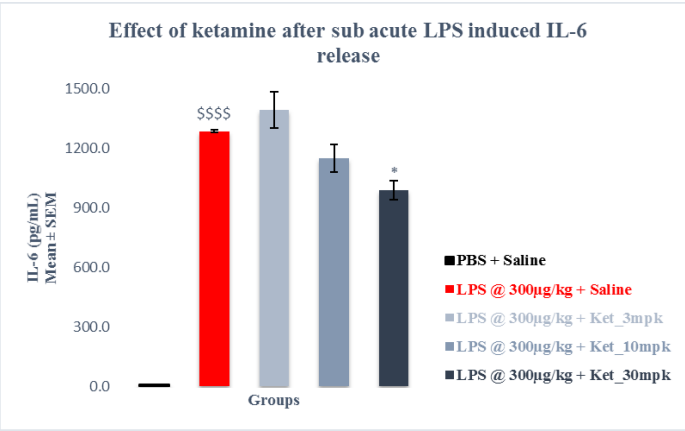


Figure 4: Rotarod test.







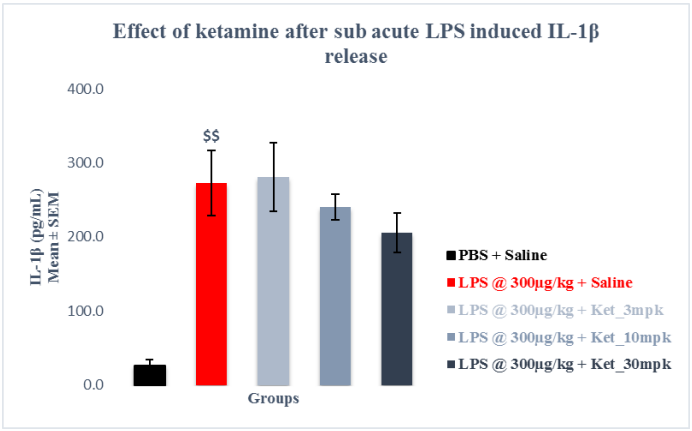


Figure 5: A-D Quantification of Plasma.

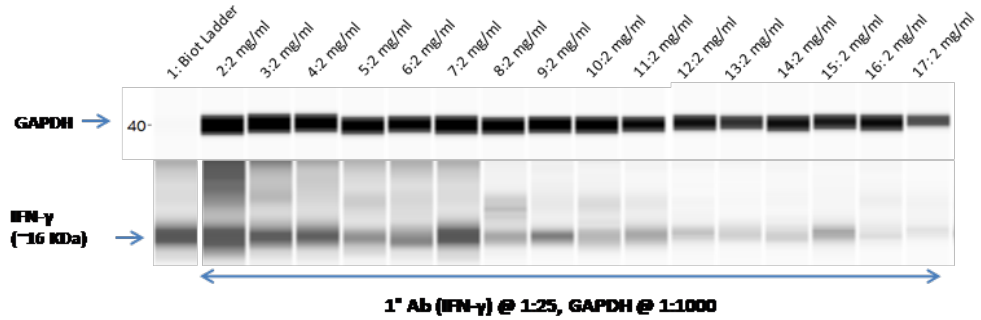


Figure 6: A, C, E, G Cytokines were measured in brain homogenate using western blot performed with automated Jess system (ProteinSimple). Chemiluminescence WB image (2-5 LPS, 6-9 Ket_3 mpk, 10-13 Ket_10 mpk, 14-17 Ket_30 mpk).
B, D, F, H Peak area obtained from each band for cytokine and GAPDH was used to calculate ratio. Percentage inhibition in expression of cytokine was calculated for each sample in comparison to LPS control group. IFN-γ [A, B], IL-6 [C, D], TNF-α [E, F], IL-1β [G, H].
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 when Ket dosed groups compared with LPS control group.

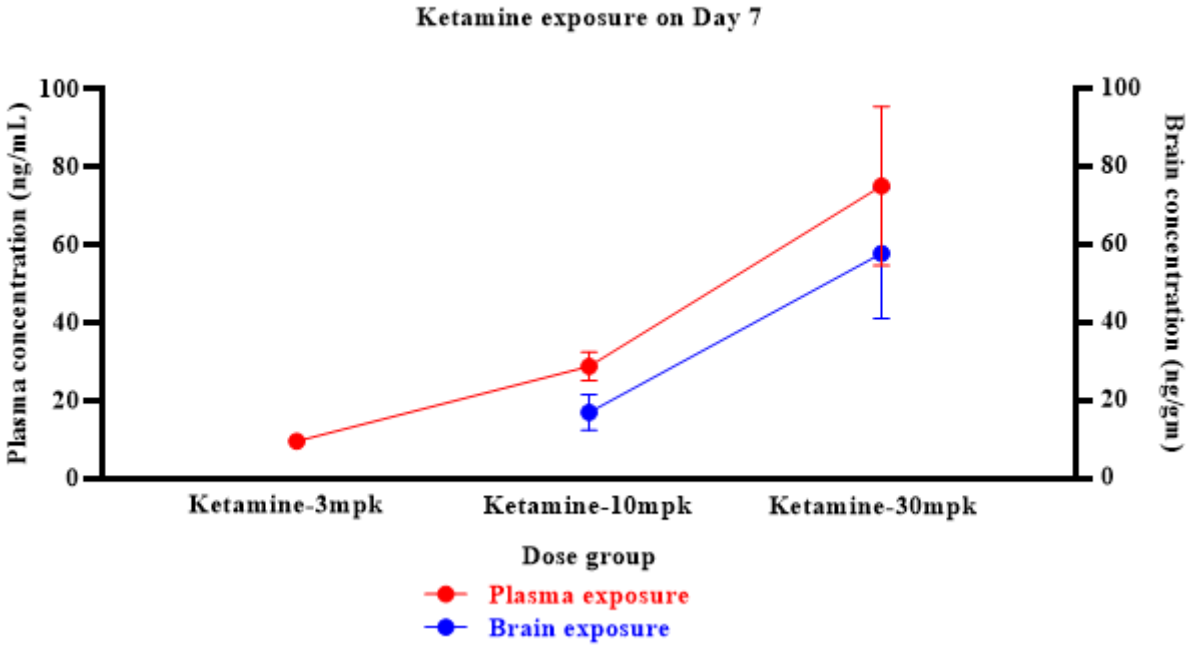


Figure 7: Pharmacokinetics analysis of Ket level measured in plasma and brain. On Day 7, mice were sacrificed at 1 hr after Ket injection.

and 150 min after injection, whereas Ket at 30 mpk dose didn't produce motor incoordination effect.

Effect of Ket on LPS induced IFN-γ in plasma and brain

The plasma level of IFN-γ protein increased in response to LPS challenge, measured 24 hr after last LPS injection (Figure 5A, Table 5). Ketamine dosed at 30 mpk significantly inhibited (79%) plasma IFN-γ level as compared with the LPS group.

The IFN-γ expression in brain (Figure 6A) and the peak area (Figure 6B) were significantly inhibited (p<0.001) by the Ketamine dosed at 10 mpk (34%) and 30 mpk (36%).

Effect of Ket on LPS induced IL-6 in in plasma and brain

The plasma level of IL-6 protein increased in response to LPS challenge, measured 24 hr after last LPS injection (Figure 5B, Table 5). Ket dosed at 30 mpk significantly inhibited (23%) plasma IL-6 level as compared with the LPS group.

Table 5: Effect of Ket in LPS induced changes in cytokine expression in plasma.

Groups	IFN-γ (pg/mL) Avg±SEM	IL-6 (pg/mL) Avg±SEM	TNF-α (pg/mL) Avg±SEM	IL-1β (pg/mL) Avg±SEM
PBS+Saline	161.6±13	9.6±0.8	1.6±0.1	26.6±7.3
LPS @ 300 µg/kg+Saline.	514.1±69 $^{\$\$}$	1285.4±6.3	98.4±3.7	272.8±44.2$^{\$\$}$
LPS @ 300 µg/kg+Ket @ 3 mpk.	495.0±8.7	1391.3±92.1	101.7±2.0	280.7±46.2
LPS @ 300 µg/kg+Ket @ 10 mpk.	462.0±14.3	1148.9±69.3	98.0±1.7	240.7±17.6
LPS @ 300 µg/kg+Ket @ 30 mpk.	235.4±101.6 *	988.4±45.9 *	73.8±2.6 ****	205.9±26.8

$^{\$\$}p<0.01$ and $p<0.0001$ vs. Saline; $^{*}p<0.05$ and $^{****}p<0.0001$ vs LPS. One-way ANOVA followed by Dunnett's post hoc test.

Table 6: Exposure for Ket in plasma and brain tissue of mice.

Dose (mpk)	Brain (ng/g) Avg±SEM	Plasma (ng/mL) Avg±SEM	B/P ratio
Ket_3	BLQ	9.5±1.4	-
Ket_10	16.9±4.0	28.7±3.6	0.70
Ket_30	57.8±16.6	75.1±20.4	0.82

A trend of inhibition in brain IL-6 expression (Figure 6C) and the peak area (Figure 6D) were observed with Ket.

Effect of Ket on LPS induced TNF-α level in in plasma and brain

The plasma level of TNF-α protein increased in response to LPS challenge, measured 24 hr after last LPS injection (Figure 5C, Table 5). Ket dosed at 30 mpk significantly inhibited (25%) plasma TNF-α level as compared with the LPS group.

TNF-α expression in brain (Figure 6E) and the peak area (Figure 6F) were significantly inhibited ($p<0.01$) by Ket dosed at 10 mpk (45%) and 30 mpk (41%).

Effect of Ket on LPS induced IL-1β in in plasma and brain

The plasma level of IL-1β protein increased in response to LPS challenge, measured 24 hr after last LPS injection (Figure 5D, Table 5). Ket showed a trend of inhibition in plasma IL-1β level.

IL-1β expression in brain (Figure 6G) and the peak area (Figure 6H) were significantly inhibited by Ket dosed at 10 mpk ($p<0.05$, 15%) and 30 mpk ($p<0.0001$, 37%).

Exposure of Ket in plasma and brain

On Day 7, Ket was dosed i.p and 1 hr post injection, pharmacokinetics analysis for the Ket level was performed in plasma and brain. Mice were sacrificed on Day 7, 1 hr post dosing.

Ket level measured in plasma at 3 mpk, 10 mpk and 30 mpk were 9.5 ng/mL, 28.7 ng/mL and 75.1 ng/mL respectively and in brain at 3 mpk, 10 mpk and 30 mpk were found to be BLQ, 16.9 ng/g and 57.8 ng/g respectively. The brain/plasma ratio of Ket at 10 and 30 mpk were calculated as 0.70 and 0.82 respectively (Figure 7, Table 6).

DISCUSSION

The main objective of the present study was to establish a potential depressant-like behavior in mice after multiple injections of LPS, which was confirmed by increased immobility time in the FST and reduction in the preference for a sucrose solution.[18] Understanding of major depression in rodents by FST and SPT provides translational relevance for human subjects. Mice exposed to LPS exhibited illness behaviour and lost body weight.[19] Hence, we tried to establish a depression condition with tweak in dose for LPS where there is no significant change in B/W, which is generally considered to be a good measure for well-being.[20] Female mice were not used as timing in the oestrus cycle, can also modulate the murine response to an immune challenge.[21] A depressant like animal model was identified after 6 days of low dose of LPS injection (300 µg/kg) where there was a significant drop in B/W up to Day 5 and animals recovered from B/W loss on Day 7. At 24 and 48 hr following LPS, there was an increase in Iba-1 immunoreactivity and de-ramified microglia and antidepressants drug can modulate it.[22]

We preferred Ket over other anti-depressant drugs as at low doses it relieved spontaneous pain and at higher doses, it treats pain which measured by filaments and cold hypersensitivities stimuli. Ket at 10 mpk is known to alleviate depression without causing psychomotor side-effects.[9] At the same time, Imipramine upon acute dosing is ineffective in human depression and LPS induced suppression of saccharin preference.[23] At higher doses Ket has the onset of sedative effects,[24] so we investigate the adverse effect for the Ket (30 mpk, i.p) in the mouse rotarod test.

Other NMDA receptor antagonists, such as (+)-MK-801 (0.3 mpk) and CGP 37849 (5 mpk), had shown anti-anhedonia effects with improved sucrose intake in another sensitive and appropriate chronic mild stress model.[9]

Gram-negative bacterial LPSs are known to stimulate the production of proinflammatory cytokines mainly through Toll-Like Receptor (TLR) 4 and nuclear factor-κB (NF-κB).[25] Six days after intraperitoneal injection of LPS, we observed an upregulation of proinflammatory cytokines in plasma and brain, which was measured by ELISA and simple WES method respectively. The plasma expression of IFN-γ, IL-6, TNF-α and IL-1β increased intensively in LPS group. A significant inhibition in IFN-γ (79%), IL-6 (23%), TNF-α (25%) was observed with Ket (30 mpk) while non-significant inhibition of IL-1β (27%) was observed. Similarly, quantification of IFN-γ, IL-6, TNF-α and IL-1β in brain homogenate was determined by western blot performed with automated Jess system. GAPDH was chosen as an internal control and for normalization, because this gene was stably expressed in mouse brain.[26] Subanesthetic Ket dosed at 10 and 30 mpk reduced 34% and 36% of IFN-γ expression in brain compared to LPS group.

IL-6, which is involved in pathological neurodegenerative disorders, may affect brain function and was inhibited by 31% and 38% with Ket dosed at 10 and 30 mpk respectively.

TNF-α has a complex mechanism in the CNS, where it plays a dual role in producing either neurodegeneration or neuroprotection. Ket dosed at 10 and 30 mpk reduced 45% and 41% of TNF-α expression in brain homogenate. IL-1β, which is important for learning, memory and cognition process, was inhibited by 15% and 37% with Ket dosed at 10 and 30 mpk respectively.

The half-life of Ket in male C57BL/6 mice is ~30 min, which indicates a possible high clearance from body.[5]

Pharmacological responses are driven by the total concentration of a drug at the site of action, such as the brain.[27]

On Day 7, 1 hr after the administration of Ket the exposure in brain and plasma of male C57BL/6 mice was analysed and the B/P ratio for 10 mpk and 30 mpk was calculated to be 0.70 and 0.82 (Table 4). Further detailed studies investigating its sustained antidepressant effects on earlier time points are needed.

Thymus weight was significantly decreased in the early stage[28] (after 6 days of LPS injection), but recovered by the late stage (after 17 days of LPS injection), in a murine model of LPS induced inflammation (Data not shown here). However, it is possible that the dose of Ket employed was not sufficient to alter the thymic weight during this tested condition. Similarly, mice developed splenomegaly after systemic inflammation in LPS group,[29] which was also not altered by any dose of Ket tested in this experimental paradigm.

CONCLUSION

The current study uses peripheral low dosage of LPS-induced neuroinflammation as a proxy for depressive symptoms in human. Our results shown that sub-acute low dose LPS injection results in depression-like symptoms with altered behavioural measures including FST and reduced SPT in mice, despite no significant change in B/W. When compared to the LPS group on Day 7, daily Ket administration improved antidepressant-like behavioural despair (i.e., decreased immobility time in FST and increased sucrose preference). A correlation was seen between Ket's anti-inflammatory effects, having a good B/P ratio at 10 mpk and 30 mpk, as well as an anti-depressant-like effect.

To our knowledge, this is the first Ket report on low dose LPS-induced depression, as well as the more sensitive and appropriate one. These results divulge new insights to the neuroinflammation inhibition.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ANOVA: One-way analysis of variance; **BLQ:** Below the limit of quantification; **B/P ratio:** Brain/plasma ratio; **B/W:** Body weight; **CRF:** Corticotropin-releasing factor; **EDTA:** Ethylenediaminetetraacetic acid; **ELISA:** Enzyme-linked immunosorbent assay; **FST:** Forced swim test; **IAEC:** Institutional Animal Ethics Committee; **IFN-γ:** Interferon gamma; **IL-1β:** Interleukin-1β; **IL-6:** Interleukin 6; **i.p.:** Intraperitoneal; **Ket:** Ketamine; **LC-MS/MS:** Liquid Chromatography Tandem Mass Spectrometry; **LPS:** Lipopolysaccharide; **mpk:** mg/kg; **NF-κB:** Nuclear factor-κB; **NMDA:** N-Methyl-D-aspartic acid; **SEM:** Standard Error of the Mean; **SPT:** Sucrose Preference Test; **TLR4:** Toll-like receptor 4; **TNF-α:** Tumor Necrosis Factor Alpha; **W/V:** Weight/Volume.

SUMMARY

A depressant like animal model was observed after 6 days of LPS injection, where LPS did not promote body weight loss. Due to good brain penetration, Ketamine showed significant efficacy in brain cytokine inhibition, suggesting that B/P ratio may be a potential factor to show better neuroinflammation inhibition.

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