Current Trends in Biotechnology and Pharmacy
Vol. 16 (Supplementry Issue 1) 102-110, 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2022.2s.36

Protective Effect of Non-Selective COX Inhibitor On Lipopolysaccharide-Induced Neuroinflammation in Rats Through NF-κB Pathway

Prakash R[1*], Ramanathan M[2]

[1]Crescent School of Pharmacy, B.S. Abdur Rahman Crescent Institute of Science and Technology, Vandalur, Chennai-600048, Tamil Nadu, India
[2]PSG College of Pharmacy, Peelamedu, Coimbatore-641004, Tamil Nadu, India.
*Corresponding author : prakasheeba@rediffmail.com

Abstract

Inflammations have been implicated in a variety of acute as well as chronic neurologic and neurodegenerative disorders. In the current work, we have investigated that selective or non-selective COX inhibitors can reduce the inflammatory response induced by bacterial endotoxin Lipopolysaccharide (LPS). Rats were pretreated with COX -1 selective inhibitor (Resveratrol 10 and 20mg/kg), COX-2 inhibitor (Celecoxib 10 and 20mg/kg) or non selective COX inhibitors (Aspirin 10 and 20mg/kg) for 15 days followed by single challenged LPS (100µg/kg., i.p.). In behavioural tests, pretreated with non-selective COX inhibitors reversed the locomotor and exploratory behavior produced by LPS in the open field test and actophotometer. Reverse transcriptase-polymerase chain reaction analysis indicated that aspirin significantly attenuates LPS induced upregulated the tumour necrosis factor-α (TNF-α) and inducible nitric oxide synthases (iNOS) expression in rat's brain. In histopathological analysis, aspirin reversed the neuronal damage induced by LPS when compared to selective COX inhibitors. Mechanistically, aspirin strongly attenuated the activation of transcription factor nuclear factor kappa B (NF-κB). Having taken the data together, we strongly suggested that a non-selective COX inhibitor is useful for mitigating neuroinflammatory conditions by block inflammatory cytokines via the nuclear factor kappa B pathway.

Keywords: Inflammation, neurodegenerative disorders, Lipopolysaccharide, cytokines, RT-PCR

Introduction

Inflammation is a protective reaction involving immune cells, blood vessels, and molecular mediators that occurs when bodily tissues are exposed to damaging stimuli such as pathogens, damaged cells, or irritants [1]. Activation of the immune system happens when an organism is either rarely or inadvertently exposed to a pathogen. Change happens due to the central nervous system's activation, including decreased locomotor activity and exploratory behavior raised in concentrations of pro-inflammatory cytokines [2]. Collectively, this behavioral alteration mediated by immune system activation is termed as sickness behavior [3]. In rodents, an adaptive behavior change is easily induced by LPS peripherally. LPS is a bacterial endotoxin that is found in Gram-negative organisms in the outer cell wall. Microglial and astrocytes stimulation that responds to LPS can upregulate the pro-inflammatory cytokines and oxidative damage [4].

AChE inhibitors (AChEIs) are currently the first-line medications used to treat Alzheimer's disease around the world [5]. Many

Current Trends in Biotechnology and Pharmacy
Vol. 16 (Supplementry Issue 1) 102-110, 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2022.2s.36

studies have shown that chronic therapy with non-selective COX inhibitors has a protective effect in neurodegenerative diseases like Alzheimer's disease, Parkinsonism disease etc. The rate-limiting enzyme is cyclooxygenases (COX) which can transform arachidonic acid into prostaglandins, and it was proposed to play a considerable function in brain-related dysfunction. COX is present in two different isoforms, such as COX-1 & 2, respectively. Both COX 1 and 2 isoforms can vary in location, mechanism and preferential coupling to upstream and downstream in the central nervous system [6-8]. The COX-1 enzyme is constitutively expressed in many tissues, and this isoform is responsible for maintaining the prostaglandin synthesis, with diverse COX-2 being an inducible enzyme in neurodegenerative conditions expressed both in neurons and glial cells [9]. Hence, it is not surprising that enormous research proposed that COX-2 enzyme inhibition has both anti-inflammatory and neuroprotective actions [10-12], arising some doubt in selective COX- 2 inhibitors possess potential adverse effects. According to the recent study indicates that COX- 1 isoenzyme act as a very important responsibility in inflammatory response due to activated microglia cells [13-15]. In response to systemic lipopolysaccharide administration, COX-1, which is predominantly present in Virchow-Robin spaces in the brain mediated immunity to brain signalling [16].

Hence, we suggest that reconsidering the rampant hypothesis, COX enzyme is stimulated in peripheral administration of bacterial endotoxin. COX-2 inhibitor is well documented for anti-inflammatory medication and COX-1, predominately present in microglia cells, has a vital role in the inflammatory cascade. With this background, the current research was investigating the role of aspirin, resveratrol and celecoxib on LPS induced behavioral, biochemical alterations and neuronal damage in rats.

Materials and Methods

Research design: Male Wistar rats weighing between 160-180gms bred in PSG Institute of Medical Science and Research animal house (PSGIMS&R) were used. Animals were brought to the lab and allowed to acclimatize for 14 days. They were housed under normal conditions to maintain the temperature (25±2°C), 50% humidity, 12 hrs dark/light cycle and provided with feed pellets and water *ad libitum.* The rats were randomly split into eight groups of six in each as follows. Control group (Normal saline group n=6). LPS group (100µg/kg., i.p.), + Saline. Aspirin plus LPS treated group (10mg/kg + 100µg/kg., i.p), Aspirin plus LPS treated group (20mg/kg + 100µg/kg., i.p), Resveratrol plus LPS treated group (10mg/kg + 100µg/kg., i.p), Resveratrol plus LPS treated group (20mg/kg + 100µg/kg., i.p), celecoxib plus LPS treated group (10mg/kg + 100µg/kg., i.p), celecoxib plus LPS treated group (20mg/kg + 100µg/kg., i.p). The research work was authorized by the committee of the institutional level and proceeded as per the guidelines of CPCSEA, New Delhi. (IAEC No. Proposal No. 170/dated 17/11/2012). The experimental design of behavioral study and drug administration was clearly shown in Figure 1.

Drugs and chemicals: Celecoxib, Aspirin, Resveratrol, LPS were obtained by Sigma Aldrich Company USA. Analytical grade standard was used for the other chemicals and reagents in the current research.

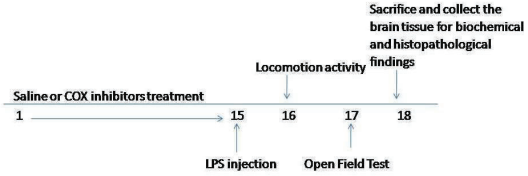

Figure 1: The experimental design of behavioral study and drug administration

Actophotometer: An actophotometer was used to determine the locomotor behaviour of the rats. In the actophotometer cage, the animals were placed individually for 3 mins for habituation, prior day to the main study. There-

after, locomotor activity was counted for 5 min duration by placing the rats individually in the actophotometer. Ambulatory behavior was documented for 5 minutes (17).

Exploratory behavior: The exploratory behavior test was assessed in the open field apparatus. The apparatus consists of a 72×72 cm square area with 36 cm height. Except for six millimeter plain lines, which divide the floor into 9 squares of the same area, the entire instrument was black painted. The experiment was done in the darkroom with low-intensity light (45W), and the open field apparatus was kept above the floor level approximately 50 cm. The rats were positioned and observed for 5 minutes in the central square, and the following behaviors were recorded. Central Square entries: The rats crossed the white lines with all four paws into the central square; Time spent in the central square, Locomotion: movement of the rats crossing with all the four paws; rearing: rats stood on its hind limbs. The apparatus was washed with 5 percent alcohol between tests [18].

Isolation of brain: Following behavioral experiments, the rats were sacrificed by cervical decapitation and brain tissues were preserved. The isolated brain tissues were used for proin flammatory markers estimation and neuronal damage analyses (n=3).

Reverse ranscriptase PCR: TRIzol reagent was used to isolate the RNA from brain tissue. For PCR amplification, approximately 100 ng RNA was added in a single-stage RTPCT kit. (Takara, USA). GAPDH amplification was used as a housekeeping gene. The primers used were TNF- α- forward 5′- CATCCGTTCTCTACCCAGCC-3′ and reverse 5′- AATTCTGAGCCCGGAGTTGG -3′ (146 bp); iNOS- forward 5′- ACACAG TGTCGCTGGTTTGA -3′ and reverse 5′- AACTCTGCTGTTCTCCGTGG -3′ (135 bp); GAPDH-forward 5′- ACCACAGTC CATGCCATCAC-3′ and reverse 5′- TCC ACCACCCTGTTGCTGTA -3’(452 bp). Two percent agarose gel was electrophoresed on the amplification materials were visualised by ethidium bromide staining, and photographed using an image analysis method.

Western blot analysis: Isolated brain tissue was washed with 500µl lysis buffer and homogenized with normal saline. Homogenized content was transferred into microcentrifuge tubes and sonicated for 10s followed by 15 min centrifugation at 20,800 rpm. According to the Bradford method, protein concentrations were quantified. The lysis buffer homogenates are mixed with Laemmli buffer and placed in a boiling water bath for 5 min. Proteins were separated in ten percent polyacrylamide gel, and protein was transferred to nitrocellulose membrane. The membrane was then probed with antibodies of antiphospho-p65 (NF-κB) followed by manufacturers' instructions. β-actin was used as an internal standard. The ECL plus Western detection kit and Gene Tools analysis programme were used to analyze [19].

Histopathological examination using cresyl violet staining: The brain cortex region was isolated and washed with 0.9% NaCl and fixed formalin solution 10% for a minimum of two days of pathological observation in brain cortex region. The paraffin portion was taken and stained with 0.1 percent cresol violet at 50 µm thickness. The brain cortex regions were examined under a binocular microscope (40X) were assessing the neuroprotection [20].

Histological assessment

As listed below, the degeneration of brain tissue was assessed based on grading:

S. No	Graded	Histopathological observations
1.	One	No significant signs of harm - (0-10%)
2.	Two	Dark neurons - (11-30%)
3.	Three	Shrunken cell bodies of dark stained cells- (31-50%)
4.	Four	Degenerative neurons with More lesions- (51-70%)
5.	Five	Appearance of necrotic cells - (71-100%)

Current Trends in Biotechnology and Pharmacy
Vol. 16 (Supplementry Issue 1) 102-110, 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2022.2s.36

Statistical study: The experimental outcome reveals that mean ± standard error is used in one way ANOVA method(Tukey multiple comparison tests). At a significance level of P<0.05, differences were considered.

Results and Discussion

Effect of COX Inhibitor on locomotoractivity

In the current investigation, we have shown that pretreated with non-selective COX inhibitors (aspirin) produced better efficacy than selective COX 1 and 2 inhibitors induced by a single exposure of LPS administered neuroinflammation in rats. Intraperitoneal administration of LPS treated rats shows evidence of decreased locomotion in the actophotometer compared to untreated rats (p<0.001). The current investigation confirmed the previous observation that an acute administration of LPS induced depressive behavior and reduction in exploratory behavior in rats [21]. In our research, we found that the behavioral changes caused by LPS were diminished with COX blockers. In aspirin and celecoxib treatment groups significantly increased the locomotor activity by dose-dependently when compared to LPS group of rats. Previous research work has supported our present study that behavioral changes caused by LPS have been attenuated by indomethacin and nimesulide [22]. Treatment of resveratrol did not significantly change the locomotion when compared to LPS groups. Our research currently indicates that the behavioral alterations caused by the LPS were better attenuated with aspirin and celecoxib. This result is consistent with the previous research work that reveals that in different behavioral evaluations following LPS administration, COX 2 was more significant than COX 1[23]. Resveratrol and celecoxib significantly decrease the locomotor activity when compared to a high dose of aspirin (P<0.001) (Figure 2).

Impact of COX inhibitor on exploratory behavior test

In the anxiety parameter measured in OFT, the times spent and the distance travelled in the peripheral line crossing of the LPS treatment rats were considerably higher against normal rats, while rats spending time and the distance travelled in the central line crossing the lipopolysaccharide treated group were substantially shorter against normal rats. LPS treated rats significantly decreased the total number of line crossings, rears, central line crossing, and the periphery compared to normal group. The previous report as shown that LPS administered group is followed by a reduction in the total travelled distance in the OF test [24].

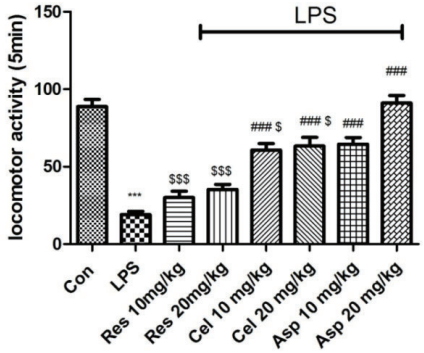

Figure 2: Effect of COX inhibitors on ambulatory behavior against LPS treated groups. Readings are expressed as mean ± standard error mean. ### p<0.001 indicates statistical implications in contrast to LPS rats. $p<0.05 and $$$p<0.001 indicates statistical implication in contrast to Aspirin 20 mg treated rats.

Aspirin and celecoxib treated rats significantly increased the total number of line crossings, rears, central line crossing and peripheral line crossing in comparison to LPS rats. Resveratrol (10mg/kg) did not reverse the LPS significantly induced the line crossing in the centre as well as periphery, rears and line crossing in the open field test, but in high dose of resveratrol shown significantly increased the number of line crossing, rears and central line crossing when compared to LPS treated rats (p<0.05). Resveratrol (10 and 20 mg/kg) treated rats had

significantly decreased the total number of line crossing, rears, central line crossing and in the periphery when compared to non-selective COX (aspirin) treated rats ($p<0.001$). Celecoxib (10 mg/kg) produced significantly decreased the total number of line crossings, rears and peripheral line crossing when compared to non-selective COX inhibitor aspirin (20 mg/kg) treated rats ($p<0.05$) (Table 1).

Effect of COX Inhibitors on pro-inflammatory markers

The impact of COX inhibitors acts on TNF-alpha and iNOS pro-inflammatory cytokines in brain homogenates assessed by RTP-CR. A significant amount of neuroinflammatory cytokine expression including COX, interleukin-6, iNOS, Tumour necrosis factor-alpha levels, were induced by LPS administration. These inflammatory cytokines are responsible for sickness behavior and affect the normal function of the brain. The release of pro-inflammatory cytokines follows the activation of microglia in LPS-stimulated cells [25]. In comparison to control rats, rats treated with LPS had higher levels of TNF- and iNOS m-RNA expression. Aspirin treated groups were significantly decrease the TNF-α ($p<0.001$) and iNOS ($p<0.001$), resveratrol (20 mg/kg) TNF- α ($p<0.05$) and celecoxib (10 and 20mg/kg) TNF-α ($p<0.05$,$p<0.01$) and iNOS ($p<0.05$) m-RNA level when compared to LPS treated rats. Recent research indicates that neuroinflammation played a key position in bipolar disorder and is also associated with inflammatory disorder. Cascade of up-regulated brain arachidonic acid and pro-inflammatory mediators; aspirin acts by dampening the up-regulated mediators [26-28]. When compared to aspirin (20mg/kg) treated group, resveratrol and celecoxib treated rats have significantly increased the pro inflammatory cytokine level (Figure 3 and 4).

Table 1: Effect of COX inhibitors on exploratory behavior against LPS treated rats

Treatment	Total No. of Line Crossing	Rearing	Central Line Crossing	Peripheral Line Crossing
Control	117.70±1.58	36.00±1.00	12.67±0.66	55.17±1.75
LPS (100µg/kg)	22.00±1.15 ***	3.83±1.22 ***	1.50±0.71***	6.17±0.65***
Res (10mg/kg)	29.50±3.53$$$	4.50±4.95$$$	2.33±3.61$$$	8.16±3.21$$$
Res (20mg/kg)	32.17±7.10#$$$	7.33±4.43# $$$	4.00±2.63#$$$	10.70±1.93$$$
Cel (10mg/kg)	73.33±1.43### $	21.00±1.84### $	7.28±2.63###	32.17±1.51###$
Cel (20mg/kg)	86.00±1.46###	23.67±1.58###	8.08±4.28###	39.17±3.22###
Asp (10mg/kg)	88.67±2.40 ###	22.17±1.37 ###	8.16±0.74###	38.17±3.33###
Asp (20 mg/kg)	92.50±2.50###	31.17±1.55###	11.17±1.47###	44.33±4.11###

Readings are expressed as mean ± standard error mean. ***$p<0.001$ states statistical implications in contrast to normal rats. ### $p<0.001$ indicates statistical implications in contrast to LPS rats. $ $p<0.05$ and $$$ $p<0.001$ indicates statistical implication in contrast to Aspirin 20 mg treated rats.

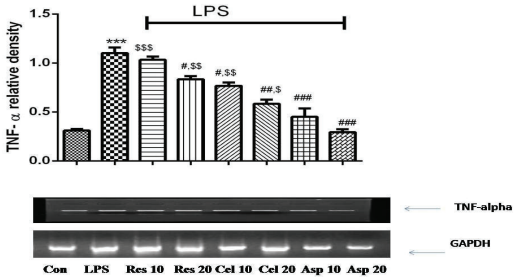

Figure 3. Effect of COX inhibitors on TNF-α mRNA expression in LPS induced neuroinflammation in rats. GAPDH was used as a housekeeping gene (internal control). Values are defined as mean ± SEM. *** p<0.001 indicates statistical implications in contrast to normal rats.

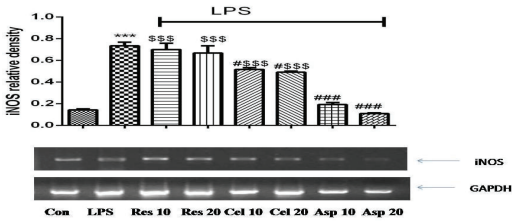

Figure 4. Impact of COX inhibitors on iNOS mRNA expression in LPS induced neuroinflammation in rats. GAPDH was used as control. Values are defined as mean ± SEM. *** p<0.001 indicates statistical implication in contrast to saline treated rats. # p<0.05 and ### p<0.001 indicates statistical implication in contrast to LPS rats. $$$ p<0.001 indicates statistical implication in contrast to Aspirin 20 mg treated rats.

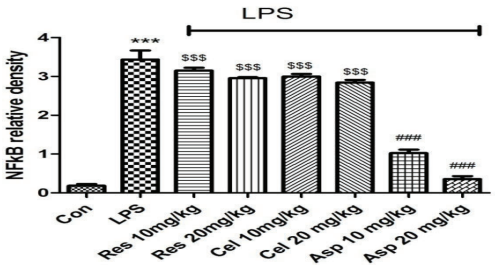

Figure 5. Effect of COX inhibitors of relative expression density of NFKB against LPS group. Readings are expressed as mean ± standard error mean. *** p<0.001 indicates statistical implication in contrast to saline treated rats. ### P<0.001 indicates statistical implication in contrast to LPS rats. $$$ P<0.001 indicates statistical implication in contrast to Aspirin 20 mg rats.

Impact of COX inhibitors against LPSinduced NF-κB p_{65} phosphorylation

Further analysis, mechanisms behind the protective effect of COX inhibitor on LPS induced the NF-κB p65 proteins were analyzed by Western blot. LPS treated rats showed a significant upregulation of NF-κB phosphorylation when compared with the normal group of rats. Pretreated with aspirin significantly decrease the p65 transcription factor protein is compared with LPS treated rats by dose-dependently (p<0.001). Previous research has supported the successful reduction of up-regulation of pro-inflammatory markers such as tumuor necrosis factor-alpha and nitric oxide synthase inducible form NSAID pretreatment induced by LPS. Correspondingly, the dampening of the nuclear factor kappa B protein activity was followed by inflammation [29]. In both, the doses, resveratrol and celecoxib did not significantly modulate the expression of NF-κB as compared to LPS group. When compared to the aspirin 20mg/kg treated group, resveratrol and celecoxib have shown significantly upregulated the expression of phosphorylated nuclear factor kappa B protein (Fig.5 and 6).

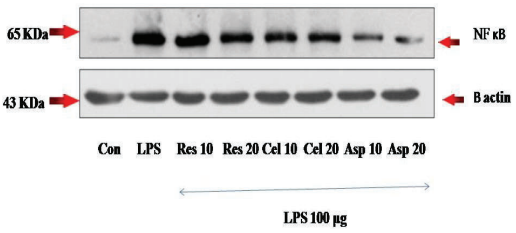

Figure 6. Effect of selective / non-selective COX inhibitors on NF κB protein expression against LPS.

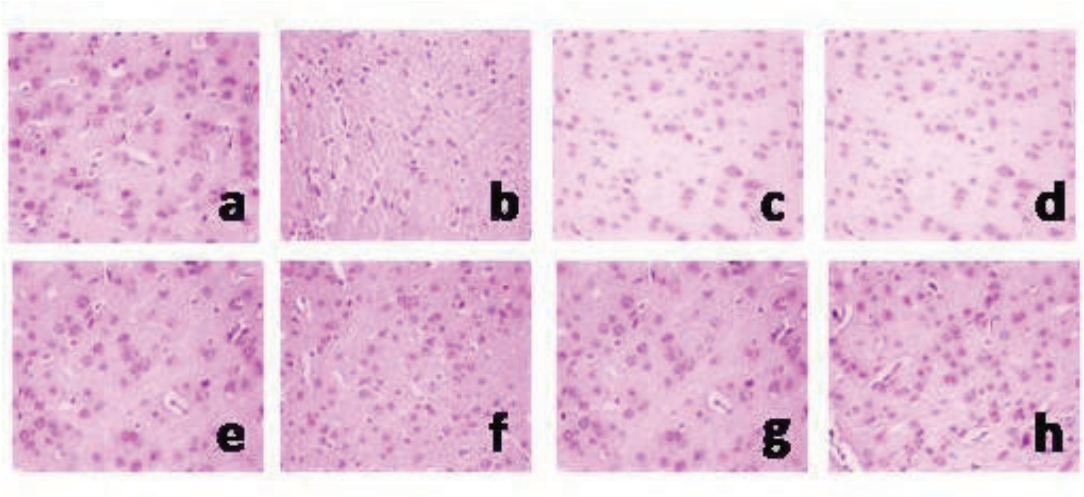

Figure 7. Neuropathological findings were observed in cortex regions using cresol violet staining procedure. (a-h) represent the different groups treated with COX inhibitor with LPS.Picture (a) the normal morphology of the pale nuclei neurons surrounded by nerve fibres, glial cells and blood vessels is shown by normal rats. Picture (b) denotes the lipopolysaccharide treated rats showing the neuronal degeneration and increased chromatolysis and pyknotic nuclei. Picture (c) denotes the resveratrol 10 mg/kg showing partially neuroprotected associated with cell bodies shrinked. Picture (d) denotes the resveratrol 20 mg/kg showing the decrease in neuronal cell degeneration. Picture (e) denotes the celecoxib 10 mg/kg showing the significant decrease in neuronal cell death with mild edematous nuclei. Picture (f) denotes the celecoxib 20 mg/kg showing the less shrinkage of neurons cell bodies and neuronal damage. Picture (g) denotes the aspirin 10 mg/kg showing the significant protected neurons without lesions. Picture (h) denotes the aspirin 20 mg/kg showing the significant decrease in cell edema and neuronal cell degeneration.

Impact of COX inhibitors against LPS induced neuronal damage

No pathological findings were observed in the region of brain cortex tissues in the normal control group. LPS treated rats have shown a reduction in neuron cells and increased the number of neurons shrunken. A previous study revealed that short-term exposure of LPS administered leads to neuronal damage [30]. In both doses, aspirin-treated rats decrease neuronal cell death, brain cell oedema, chromatolysis and pyknotic nuclei when compared to LPS treated rats. Resveratrol (10 and 20 mg/kg) showed mild cell edema and degeneration in neurons compared to LPS treated rats. Celecoxib (10 and 20 mg/kg) treated rats showed a significant reduction in neuronal cell death with mild edematous nuclei compared with LPS treated rats (Figure 7 a-h).

Conclusion

The current investigation emphasized the protective function of aspirin in neuroinflammation was mediated through NFκB mechanism. In a behavioral study, aspirin and celecoxib have shown increasing ambulatory behaviour activity, and anxiety behavior was observed. Further studies revealed that TNF-α and iNOS m RNA levels were downregulated, and neuronal damage was attenuated in the aspirin group when compared to selective COX 1 and 2 inhibitors. Furthermore, the aspirin-treated group attenu-

ated the neuroinflammation by down streaming the NFκB p65 phosphorylated protein. Among the studied drugs, aspirin was found to be better medicine in controlling the neuroinflammation induced by peripheral administration of LPS.

References

1. Bhardwaj, S., Kaur, A., Kaur, M., Singh, J.P., Kaur, J. and Nammi, S. (2021) Evaluation of Anti-Inflammatory Activity of *Tylophora asthmatica*. Curr Trends Biotechnol. Pharm. 15(6): 90-95.
2. Hennessy, M.B., Deak, T. and Schiml, P.A. (2014) Sociality and sickness: have cytokines evolved to serve social functions beyond times of pathogen exposure? Brain Behav. Immunol. 2943: 15–20.
3. Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W. and Kelley, K.W. (2008) From inflammation to sickness and depression: when the immune system subjugates the brain. Nat. Rev. Neurosci. 9: 46–56.
4. Prakash, R. (2019) Protective Effect of Resveratrol and Celecoxib on Lipopolysaccharide-induced Oxidative Stress. J. Pharm. Sci. Res. 11(6): 2440-2444.
5. Srivastava, P., Tripathi, P.N., Sharma, P. and Shrivastava, S. K. (2019) Quantitative Structure-activity Relationship based Design, Synthesis, and Evaluation of Novel Diarylether Derivatives as a potent Acetylcholinesterase inhibitor and Antioxidant to treat Cognitive dysfunctions. Curr. Trends Biotechnol. Pharm. 13(2): 124-145.
6. Bosetti, F., Langenbach, R. and Weerasinghe, G.R. (2004) Prostaglandin E2 and microsomal prostaglandin E synthase-2 expression are decreased in the cyclooxygenase-2-deficient mouse brain despite compensatory induction of cyclooxygenase-1 and Ca2+-dependent phospholipase A2. J. Neurochem. 91: 1389–1397.
7. Murakami, M. and Kudo, I. (2004) Recent advances in molecular biology and physiology of the prostaglandin E2-biosynthetic pathway. Prog. Lipid Res. 43: 3–35
8. Choi, S.H., Langenbach, R. and Bosetti, F. (2006) Cyclooxygenase-1 and -2 enzymes differentially regulate the brain upstream NF-kB pathway and downstream enzymes involved in prostaglandin biosynthesis. J. Neurochem. 98: 801–811.
9. Coray T.W. and Rogers, J. (2012) Inflammation in alzheimer disease–A brief review of the basic science and clinical literature, Cold Spring Harb. Perspect. Med. 2: a006346.
10. Bertolini, A., Ottani, A. and Sandrini M. (2002) Selective COX-2 inhibitors and dual acting anti-inflammatory drugs: critical remarks. Curr. Med. Chem. 9: 1033–1043.
11. Aid, S. Langenbach, R. and Bosetti, F. (2008) Neuroinflammatory response to lipopolysaccharide is exacerbated in mice genetically deficient in cyclooxygenase-2. J. Neuroinflammation. 5: 17.
12. Toscano, C.D., Ueda, Y., Tomita, Y.A. Vicini, S. and Bosetti, F. (2008) Altered GABAergic neurotransmission is associated with increased kainate-induced seizure in prostaglandin-endoperoxide synthase-2 deficient mice. Brain Res. Bull. 75: 598–609.
13. Choi, S.H., Aid, S. and Bosetti, F. (2009) The distinct roles of cyclooxygenase-1 and -2 in neuroinflammation: implications for translational research. Trends Pharmacol. Sci. 30:174-81.
14. Aid, S. and Bosetti, F. (2010) Targeting cyclooxygenases-1 and -2 in neuro-inflammation: Therapeutic implications. Biochimie. 93:46-5.
15. Bosetti, F. and Choi, S.H. (2010) Rethinking the role of cyclooxygenase-1 in neuroinflammation: more than homeostasis. Cell Cycle. 9:2919-20.

16. García-Bueno, B., Serrats, J. and Sawchenko, P.E.(2009) Cerebrovascular cyclooxygenase-1 expression, regulation and role in hypothalamic-pituitary-adrenal axis activation by inflammatory stimuli. J. Neurosci. 29:12970-81.

17. Reddy, D.S. and Kulkarni, S.K. (1998) Possible role nitric oxide in the nootropic and antiamnesic effects of neurosteroids on aging-and dizocilpine-induced learning impairment. Brain Res. 799(2): 215-229.

18. Carrey, N., McFadyen, M.P. and Brown, R.E. (2000) Effects of subchronic methylphenidate hydrochloride administration on the locomotor and exploratory behavior of prepubertal mice. J. Child Adolesc. Psychopharmacol. 10 (4): 277-86.

19. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72: 248–254.

20. Babu, C.S. and Ramanathan, M. (2011) Post-ischemic administration of nimodipine following focal cerebral ischemic–reperfusion injury in rats alleviated excitotoxicity, neuro-behavioural alterations and partially the bioenergetics. Int. J. Dev. Neurosci.29: 93–105.

21. Teeling, J. L., Cunningham, C., Newman, T. A. and Perry, V. H. (2010) The effect of non-steroidal anti-inflammatory agents on behavioural changes and cytokine production following systemic inflammation: Implications for a role of COX-1. BrainBehav.Immun. 24(3): 409-419.

22. de Paiva, V. N., Lima, S. N., Fernandes, M. M., Soncini, R., Andrade, C. A. and Giusti-Paiva, A. (2010) Prostaglandins mediate depressive-like behaviour induced by endotoxin in mice. Behav Brain Res. 215(1): 146-151.

23. Johnson, P. M., Vogt, S. K., Burney, M. W. and Muglia, L. J. (2002) COX-2 inhibition attenuates anorexia during systemic inflammation without impairing cytokine production. Am. J. Physiol-Endocrinol. Metab. 282(3): E650-E656.

24. Depino, A. M. (2015) Early prenatal exposure to LPS results in anxiety- and depression-related behaviors in adulthood. Neuroscience. 299: 56-65.

25. Choi, S. H., Langenbach, R. and Bosetti, F. (2008) Genetic deletion or pharmacological inhibition of cyclo-oxygenase-1 attenuate lipopoly-saccharide induced inflammatory response and brain injury. FASEB J. 22(5): 1491-1501.

26. Poletti, S., Leone, G., Hoogenboezem, T. A., Ghiglino, D., Vai, B., de Wit, H. and Benedetti, F. (2019) Markers of neuro-inflammation influence measures of cortical thickness in bipolar depression. Psychiatry Res. Neuroimaging. 285: 64-66.

27. Bosetti, F. (2007) Arachidonic acid metabolism in brain physiology and pathology: lessons from genetically altered mouse models J. Neurochem. 102(3): 577-586.

28. Rosenblat, J. D., Gregory, J. M. and McIntyre, R. S. (2016) Pharmacologic implications of inflammatory comorbidity in bipolar disorder. Curr. Opin. Pharmacol. 29: 63-69.

29. Liong, E. C., Xiao, J., Lau, T. Y., Nanji, A. A. and Tipoe, G. L. (2012) Cyclooxygenase inhibitors protect D-galactosamine / lipopolysaccharide induced acute hepatic injury in experimental mice model. Food Chem. Toxicol. 50(3-4): 861-866.

30. Prakash, R. and Ramanathan, M. (2013) Effect of COX-Inhibitors Attentuated LPS Induced Behavioural Alterations in Male Wistar Rats. J. Pharm. Sci. Res. 11 (5): 226-230.