

Effect of COX-Inhibitors Attenuated LPS Induced Behavioural Alterations in Male Wistar Rats

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Abstract:

Sickness behavior appears to be the expression of a central motivational state that reorganizes the organism's priorities to cope with infectious pathogens. To evaluate the effect of selective and non selective COX inhibitors in lipopolysaccharides induced sickness behaviour, rats were subjected to the forced swim test (FST), open field test, actophotometer. LPS (10mcg/kg i.p.) administration increased the immobility period in the FST, and depressed locomotor activity in the actophotometer, decreased line crossing and rearing in open field test, increased body temperature and decreased body weight. COX inhibitors are reported to be effective by reducing the risk of neuro degenerative diseases and sickness behaviours. Rats were pretreated with Non-selective COX inhibitors Aspirin (100&200mg/kg), Selective COX-1 inhibitor Resveratrol (50&100mg/kg) & selective COX-2 inhibitor Celecoxib (10&50mg/kg) for 7 days. At the last day rats were given final injection of saline or Cox inhibitors 30 min prior to LPS (10mcg/kg i.p.). After 120 min of LPS treatment determined the exploratory behaviour in open field test, depression using FST, locomotion using actophotometer, body temperature and body weight. Considering the behavioural and histopathological observations showed in the chronic treatment of Non selective COX inhibitors (aspirin) & selective COX 2 inhibitor (celecoxib) attenuated LPS induced behavioural changes but not in selective COX 1 inhibitor (resveratrol) produced by LPS. Considering that aspirin & Celecoxib attenuates LPS-induced behavioural changes, it is proposed that LPS-induced sickness behavior is dependent on the COX pathway mediate prostaglandins (PGE₂)

Keywords: Resveratrol, Celecoxib, Depression, Lipopolysaccharides Sickness behaviour

INTRODUCTION:

Lipopolysaccharides, a non-infectious component of gram -ve bacterial cell wall potent stimulus for experimental immune activation whether peripheral or central administration of bacterial endotoxin characteristically leads to behavioural changes includes reduced food and water intake, decreased exploration, decreased locomotion and increased immobility and somnogenesis (1-3) collectively these behavioural changes have been termed as Sickness behaviour. The mechanism underlying sickness behaviour have not been fully elucidated but it has been suggested that cytokines and prostaglandins are involved, IL-1 β , IL-6 & TNF α may be secreted in response to infections and endotoxemia. These behavioral responses, accompanied by neuroimmune and neuroendocrine activation, have been associated with non-specific inflammatory processes in animals. Recent evidence suggests that nonsteroidal anti-inflammatory drugs (NSAIDs) also attenuate neuroimmune and neuroendocrine activation. The term NSAID refers to a group of structurally diverse chemical compounds that share the ability to inhibit the activity of the prostaglandin (PG) biosynthetic enzymes, the cyclooxygenase (COX) isoforms 1 and 2. Prostaglandins (PGs) are major pro-inflammatory agents and the suppression of their synthesis at sites of inflammation has been regarded as primarily responsible of the beneficial properties of NSAIDs although several COX independent effects have been described in recent years. COX isoforms play a central role in the inflammatory cascade by converting arachidonic acid (AA), release from membrane phospholipids by a phospholipase A₂ (PLA₂), into prostaglandin endoperoxide H₂, which in turn is converted to bioactive prostanoids by specific terminal synthases. The two COX isoforms share

60% homology in their amino acids sequence and have comparable kinetics; however they also show individual differences. While COX-1 is a constitutive enzyme, COX-2 is induced by several stimuli. However, in the central nervous system (CNS), COX-1 and COX-2 are both constitutively expressed and COX-2 is mainly detected in the perinuclear, dendritic and axonal domains of neurons, particularly in cortex, hippocampus, amygdala and dorsal horn of the spinal cord of both rodent and human CNS [4-6]. In the CNS, COX-2 has been implicated in important physiological functions such as synaptic transmission, neurotransmitter release, blood flow regulation, and sleep/wake cycle [7-11].

Both COX-1 and COX-2 have been shown to play important roles in an inflammatory response. However, the exact role of each COX isoform in neuroinflammation is unclear. Previous reports have demonstrated that LPS-induced depressive-like behavior appears to depend on the cyclooxygenase (COX) pathway as the use of a non-steroidal anti-inflammatory drug (NSAID) (indomethacin and nimesulide) has been shown to attenuate the behavioral changes induced by LPS [12]. In another study shows selective COX-1 inhibitor, piroxicam, but not the selective COX-2 inhibitor, nimesulide reversed the LPS-induced behavioural changes, with this background the main aim of present study was conducted to clarify behavioural alterations and neuronal damage induced by LPS attenuated by either selective or non selective COX inhibitors, rats were submitted to well accepted tests to evaluate depressive-like and exploratory behaviour such as forced swim test, open field test and actophotometer (12)

MATERIALS AND METHODS:-**Animals**

Adult male wistar rats (160-180g:48 rats) were procured from central animal facilities of PSG Institute of medical sciences & research Peelamedu, Coimbatore and divided into 8 groups of 6 animals. The rats were housed in colony cages at an ambient temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and 40-65% RH with a 12:12 h L:D cycle. The animals had free access to standard pellet chow and drinking water. Behavioural studies were carried out in a quiet room between 09:00 and 11:00 a.m. to avoid circadian variation. The study was approved by institutional animal ethics committee & work was carried out as per CPCSEA guidelines, New Delhi.

Chemicals and reagents

Celecoxib & Aspirin (Madras pharmaceuticals Pvt Ltd. Chennai), Resveratrol, LPS Serotype 048:B8 (Sigma Aldrich USA) hemotoxillin & eosin were procured from Hi media, India. All other chemicals and reagents unless specified were of analytical grade.

Experimental Design:-

Male wistar rats were pretreated with Celecoxib (10&50mg/kg p.o.), Resveratrol (50&100mg/kg p.o.), Aspirin (100&200mg/kg p.o) dissolved in normal saline and given for 7 days at the last day rats were given final injection of saline or COX inhibitors 30 min prior to LPS (10mcg/kg i.p.) serotype 048:B8 (10 $\mu\text{g}/\text{kg}$ i.p.) or saline (0.9% NaCl) were administered. The behavioural parameters were performed 120 min later. These time points were chosen on the basis of previous behavioural studies. (13,14,15,16)

- Group I:- Normal control treated with saline (0.5ml-1ml i.p)
 Group II:- Treated with saline for 7 days and LPS on 7th day after 30 min of by saline administration.
 Group III:-, Resveratrol (50mg/kg p.o) for 7 days and LPS on 7th day after 30 min of Resveratrol administration.
 Group IV:-, Resveratrol (100mg/kg p.o) for 7 days and LPS on 7th day after 30 min of Resveratrol administration.
 Group V:- Celecoxib (10mg/kg p.o) for 7 days and LPS on 7th day after 30 min of Celecoxib administration.
 Group VI:- Celecoxib (50mg/kg p.o) for 7 days and LPS on 7th day after 30 min of Celecoxib administration.
 Group VII:-, Aspirin (100mg/kg p.o) for 7 days and LPS on 7th day after 30 min of Aspirin administration.
 Group VIII:- Aspirin (200mg/kg p.o) for 7 days and LPS on 7th day after 30 min of Aspirin administration

Body Weight:

On the test day 1, the body weight (BW) of each animal was measured immediately before drug treatment as well as on day 7 after injection of either saline or LPS. The changes in body weight was calculated using this formula

$$\% \text{ Decrease in Body weight} = \frac{\text{Before treatment} - \text{after treatment}}{\text{Before treatment}} \times 100$$

Rectal Temperature:[17]

By insertion of a thermo probe to a depth of 2 cm into the rectum the initial rectal temperatures were recorded on day 1 before drug treatment as well as on day 7 after injection

of either saline or LPS. The changes in rectal temperature were measured before and after treatment of drugs in all groups by using this formula

$$\% \text{ Increase in Body temperature} = \frac{\text{Before treatment} - \text{after treatment}}{\text{Before treatment}} \times 100$$

Assessment of Locomotor behavior:[18]

The locomotor activity can be easily studied with the help of actophotometer, The actophotometer consisted of a square arena (30 × 30 × 25 cm) with wire mesh bottom, in which the animal moves. Six lights and six photocells were placed in the outer periphery of the bottom in such a way that a single rat can block only one beam. The movement of the animal interrupts a beam of light falling on a photocell, at which a count was recorded and displayed digitally. The locomotor activity was measured for a period of 10 min. Technically its principle is that, a photocell is activated when the rays of light falling on the photocells are cut off by animals crossing the beam of light. As the photocell activated, a count is recorded. 4 hours after the administration of the vehicle or Standard or test each rats was tested for activity for 10 min.

Open field exploratory behaviour:

Locomotor activity was quantified for 5min in an open field, which was a white Plexiglas box 60cm×60cm with its floor divided into 16 squares. Previous studies have indicated that this period was sufficient to indicate differences between treatment groups. Furthermore, if the test was longer than 5min, the mice habituated to the apparatus, thereby decreasing differences between groups. Four squares were defined as the centre and the 12 squares along the walls were considered the periphery. Each mouse (n=10 per group) was gently placed exactly in the centre of the box and activity was scored as a line crossing when a mouse removed all four paws from one square and entered another. Line crossings among the central four squares or among the peripheral 12 squares of the open field counted separately (Dunn and Swiergiel, 2005).

Forced swim test:[19]

Rats were allowed to swim individually in a chamber (45 X 12 X 45 cm) containing fresh water $25^{\circ}\pm 2^{\circ}\text{C}$ of height 35 cm, so that the rat could not touch the bottom of the cylinder with its limb or tail or climb over the edge of the chamber. Two swim sessions were conducted an initial 15 min pretest followed by 6 min test 24 h later. The period of immobility after an initial 2-3 min period of vigorous activity was recorded. A rat was considered to be immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 min of a total 6 min test. Rats were then allowed to dry in a pre-warmed enclosure ($\sim 32^{\circ}\text{C}$) before being returned their home cage. All the behavioural experiments were carried out between 0900-1100h by blind observer with recording.

Statistical analysis

Data was expressed as mean \pm SEM mean difference the behavioral parameters were analysed by one way ANOVA followed by tukey multiple comparison

test.statistical analysis was performed using GraphPad Prism, 5.01 (San Diego, US). P <0.05 was fixed as the statistical significance criterion.

RESULTS:

Effect of Resveratrol, Celecoxib And Aspirin on Body Weight:

There was a decreased body weight in LPS rats pretreated with saline when compared to control rats.Pretreatment with Aspirin (100&200mg/kg),celecoxib (10&50mg/kg) and resveratrol (50&100mg/kg) showed increased body weight when compared to LPS treated rats.(Table.1)

Effect of Resveratrol, Celecoxib And Aspirin on Rectal Temperature:

LPS treated rats showed significantly increased rectal temperature recorded on telethemometer when compared to control rats(P<0.001).Pretreatment with Aspirin (100&200mg/kg),celecoxib (10&50mg/kg) and resveratrol (50&100mg/kg) showed significantly decreased in rectal temperature recorded on telethemometer when comparison to LPS treated rats (Table.1)

Effect of Resveratrol, Celecoxib And Aspirin on Locomotor Activity:

LPS treated rats significantly decreased locomotion in actophotometer when compared to control rats(P<0.001).Aspirin (100&200mg/kg),Celecoxib (10mg/kg &50mg/kg) and Resveratrol (100mg/kg)shows increased locomotion when compared to LPS treated rats .Resveratrol (50mg/kg) did not produce significant in locomotion in actophotometer . (Table.1)

Effect of Resveratrol, Celecoxib And Aspirin in open field test

LPS significantly decreased the number of line crossing in the center and in the periphery as well as the total number of line crossing and number of rears when compared to control rats (P<0.001).Pretreatment with Aspirin(100&200mg/kg), Celecoxib(10&50mg/kg)significantly reversed the LPS induced decreases number of line crossing in the center (P<0.01,P<0.001,P<0.001and P<0.01) and in periphery (P<0.05,P<0.001,P<0.01 and P<0.05) as well as the total number of line crossing (P<0.001)and rearing (P<0.001) but resveratrol (50mg/kg and 100mg/kg) did not reversed the LPS induced decrease number of line crossing in center, periphery as well as the total number of line crossing and rearing. (Fig 1. a, b, c, d)

Effect of Resveratrol, Celecoxib And Aspirin in Forced Swimming Test

There was a significant increase in immobility period 120 min after administration of LPS (P<0.001) in rats pretreated with saline.Pretreatment with Aspirin (100&200mg/kg) and celecoxib (10&50mg/kg) significantly reversed the LPS mediated increase in the immobility period (P<0.001). Resveratrol (50&100mg/kg) did not produce significant during the forced swim test (Table.1)

DISCUSSIONS

The present study confirmed the previous observations that LPS can induce sickness behaviours. These results proved the evidence that the synthesis of prostaglandins was necessary for the development of depressive-like and exploratory behaviours in rats,Prostaglandins PGE2 is being considered the major metabolite synthesized by COX .So COX inhibitors also abolished the response and decreased LPS-induced behaviours.

LPS administration increased in body temperature,decreased in body weight increased the time spent floating in the FST, and depressed locomotor activity in the actophotometer ,decreased line crossing in the center and periphery as well as the total number of line crossing and rearing in open field test. Jain et al. [20] reported that systemically administered LPS to mice increased floating in the FST. Dunn and Swiergiel previously reported similar effects is the TST, FST and open field after LPS (1–5mcg/mouse) or IL-1(100–1000ng/mouse) injection.

A systemic challenge of LPS not only results in cytokine production, but also in increased production of lipophilic molecules including prostaglandins (PGE2), leukotrienes, and thromboxanes,which can all contribute to behavioural changes. Apart from neutralising COX activity, it has been described that indomethacin and ibuprofen are potent inhibitors of thromboxanes (Higgset al., 1986). Furthermore, indomethacin and ibuprofen can directly bind and activate PPAR α that leads to an anti-inflammatory response that is independent of COX (Lehmann et al., 1997). indomethacin more potent than ibuprofen (Botting, 2006; Gierseet al., 1999). We observed that non selective NSAID is a more potent inhibitor of LPS-induced behavioural changes and PGE2 production in the brain.

Table No.1 Effect of cox inhibitors attenuated on LPS induced behavioral parameters in male wistar rats

| Groups | Treatment | % Decrease in body weight | % Increase in rectal temperature | Locomotor activity (5 min) | Floating time (in sec) |
|--------|--------------------------|---------------------------|----------------------------------|----------------------------|------------------------|
| I | Control | 0.23±0.15 | -0.18±0.19 | 88.83±4.58 | 22.33± 2.155 |
| II | LPS10µg/kg i.p. | -0.00±0.13 | 1.22±0.25*** | 26.33±3.55*** | 120.5± 22.15*** |
| III | Celecoxib 10mg/kg p.o | 0.14±0.09 | -0.09±0.16### | 64.50±4.25#### | 127.0± 4.058#### |
| IV | Celecoxib 50mg/kg p.o | 0.16±0.10 | -0.22±0.08### | 91.00±4.96#### | 101.0± 3.596#### |
| V | Resveratrol 50mg/kg p.o | 0.18±0.11 | 0.23±0.22# | 47.67±6.59 | 139.0± 4.359 |
| VI | Resveratrol 100mg/kg p.o | -0.19±0.22 | 0.17±0.23## | 65.67±4.20## | 135.8± 3.842 |
| VII | Aspirin 100mg/kg p.o | 0.00±0.12 | 0.17±0.13## | 65.83±5.13#### | 59.67± 9.358#### |
| VIII | Aspirin 200mg/kg p.o | 0.00±0.12 | 0.00±0.12### | 64.50±5.88#### | 38.17± 4.354#### |

Values expressed in mean±SEM:significance with tukey's test following one way ANOVA is indicated symbol denote the significance level :*p<0.05,**P<0.01 and P<0.001 when compared with control groups(LPS plus Saline): #p<0.05,##P<0.01 and ####P<0.001 when compared with the (LPS plus vehicle)

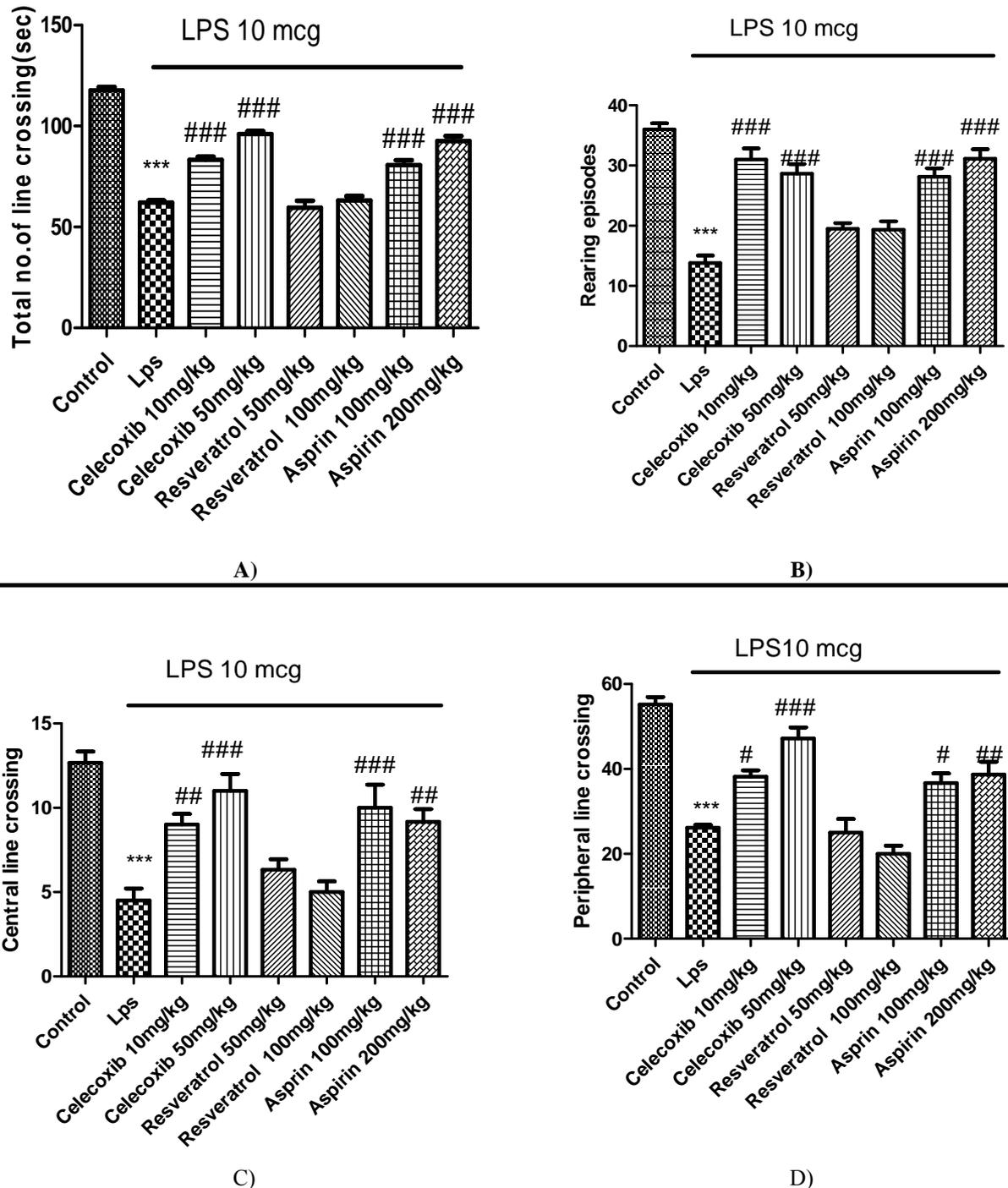


Fig 1 A,B,C,D..Effects of Pretreatment with Non selective cox inhibitors Aspirin (100&200mg/kg), Selective cox1 inhibitor Resveratrol(50&100mg/kg) and Selective Cox 2 inhibitor Celecoxib (10&50 mg/kg) on and total linecrossings(A), and rearing (B) central (C), peripheral (D) in the open field test (n=6 per group), which were measured 2h after administration of either lipopolysaccharide (LPS) or saline. Each column represents the mean with S.E.M. The symbol denote the significance level : *p<0.05, **P<0.01 and P<0.001 when compared with control groups(LPS plus Saline): #p<0.05, ##P<0.01 and ###P<0.001 when compared with the (LPS plus vehicle)

COX catalyses the conversion of the lipid metabolites arachidonic acid to PGs, and plays a key role in several physiological and pathological processes. COX-1 is constitutively expressed in many cell types(Funk et al., 1991), and responsible for the production of PGs that are necessary for the regulation of physiological functions (Crofford, 1997). COX-2 is induced by diverse

inflammatory stimuli (DuBois et al., 1997; Mitchell et al., 1994; O’Sullivan et al., 1992) and is responsible for the production of PGs in inflammation (Vane1994). It is generally believed that LPS, or cytokines induce COX-2 and mPGES-1 expression in cerebral endothelial cells, with subsequent PGE2 production in the CNS leading to both fever and behavioural changes. (DuBois et al., 1997; Ek et

al., 2001;Engblom et al., 2002; Mitchell et al., 1994; O'Sullivan et al., 1992;Yamagata et al., 2001). In this study, we show that changes in FST, locomotion, and open field test dependent on COX pathway and correlate with systemic production of PGE₂, not cytokines. Previous report shows that circulating and brain PGE₂ levels increased significantly after systemic LPS challenge starting at 30min and peak at 2h [20]. In addition, the pharmacological inhibition of cyclooxygenase, using indomethacin and nimesulide, effectively attenuated the depressive-like behaviour LPS-induced inflammation. There are a number of studies that suggest a role for COX-1 & COX-2 in regulating brain inflammatory responses.

Communication between the peripheral immune system and the brain is a well described phenomenon. Despite numerous studies, the biological mechanism(s) underlying these behavioural changes are still not fully understood. To further study the mechanisms underlying these observations, we pretreated rats with a selection of widely used anti-inflammatory drugs, including aspirin (non-specific COX inhibitor), celecoxib (highly selective COX-2 inhibitor), resveratrol (COX1 inhibitor), and assayed the behavioural changes following a peripheral administration of LPS. Our study showed that pretreatment with COX inhibitors blocked behavioural changes induced by LPS immobility period in the FST, locomotion in actophotometer and exploratory behaviour in open field test. Previous report shows that circulating and brain PGE₂ levels increased significantly after systemic LPS challenge starting at 30min and peak at 2h[21]. In addition pharmacological inhibition of non selective COX, using Aspirin, effectively attenuated the immobility period in FST, locomotion in actophotometer and line crossing in the center and periphery as well as total number of line crossing and rearing in the open field test response to systemic LPS-induced inflammation, while selective COX 2 inhibitors celecoxib (10 mg/kg & 50mg/kg) attenuated the immobility period in FST locomotion in actophotometer and line crossing in the center and periphery as well as total number of line crossing and rearing in the open field test, selective COX 1 inhibitor Resveratrol(50 mg/kg & 100 mg/kg) had no effect in forced swim test, open field test but only increase locomotion in actophotometer in resveratrol (100mg/kg) to systemic LPS-induced inflammation. One common effect of LPS exposure is reduced body weight. Decreases in body weight may arise due to reduced food intake, but have also been predicted to arise because of altered metabolic activity [22]. LPS-induced loss of body weight was significantly greater in saline-treated rats versus non-selective COX inhibitors, selective COX2 inhibitor not in selective COX1 inhibitors. This finding compliments work showing that the NSAID zaltoprofen attenuated decreased body weight and sickness behavior induced by concanavalin-A (an activator of T-cells and cytokines) in rats [23].

In conclusion this report describes the ability of a broad spectrum NSAID & selective COX2 inhibitor attenuated LPS-induced sickness behaviour in rats. The stimulation of COX by LPS activates the COX 2-PGE₂ pathway thus

reducing the available COX enzyme. By inhibiting the LPS induced production of COX 2 with a broad spectrum NSAID & selective COX 2 inhibitor and thus blocking the stimulation of the AA-PG pathway.

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