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Original Article

PROTECTIVE EFFECT OF COX INHIBITORS ON LIPOPOLYSACCHARIDE INDUCED SICKNESS BEHAVIOUR OR NEUROINFLAMMATION AND OXIDATIVE STRESS ON MALE WISTAR RATS

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ABSTRACT

Objectives: The aim of the study was to evaluate the protective effect of COX inhibitors on lipopolysaccharide induced sickness behaviour or neuro inflammation and oxidative stress on male wistar rats.

Methods: Male albino wistar rats were divided into 8 groups and each group consisting of 6 rats and drug treatment has done for one week. Control group was given normal saline daily by i. p route for 7 days. Negative control group receives saline for 6 days followed by LPS on 7th day. All other group receives Resveratrol, Celecoxib, and Aspirin for 7 days and LPS (inducing agent) was given on 7th day just before half an hour of drug treatment. After 2 hours of drug administration, the animals were subjected to behavioural testing and after analyzing behavioural parameters, the animals were sacrificed by cervical dislocation in order to perform *in vitro* studies.

Results: The results of this study showed that Celecoxib at 10 and 50 mg/kg p. o. showed no changes in body weight but there is the decrease in the temperature, increase in the locomotor activity, increase in the number of line crossing, head dipping/nose poaking, decrease in the floating time when compared to the negative control group. Resveratrol at 50 and 100 mg/kg p. o. showed a significant increase in the Superoxide Dismutase, Catalase, Glutathione Reductase, and showed significant decrease in the Lipid Peroxide and Nitric Oxide level when compared to the negative control group.

Conclusion: It was finally concluded that Celecoxib showed neuro protective activity and Resveratrol showed anti-oxidant property.

Keywords: Lipopolysaccharide, Sickness behaviour, COX inhibitors, Oxidative Stress.

INTRODUCTION

Lipopolysaccharide is as an endotoxin that elicits strong immune response in animals and it acts as prototypical endotoxin because it binds to CD14/TLR4/MD2 receptor complex that promotes the secretion of pro inflammatory cytokines IL-1β, IL-6, TNF-α especially in macrophages and β cells [1]. The circulating cytokines or other inflammatory mediators that are produced by bacterial endotoxin develops sickness behaviour syndrome as well as neuroinflammation. Sickness behaviour have been characterized by non specific symptoms such as lethargy, depression, anxiety, loss of appetite, sleepiness, hyperalgesia, reduction in grooming and failure to concentrate [2-7]. In the late phase of an inflammatory response in sickness behaviour (also seen during gram negative bacterial infections) [8], LPS creates an abundance of reactive oxygen species (ROS) primarily from macrophages and infiltrating neutrophils. ROS serve as an intracellular messenger to induce signal transduction and activate transcription factors such as nuclear factor kappa B (NFkB); therefore, production of ROS is an important one for host defense and may influence sickness behaviour via NFkB-dependent cytokine production [9]. Sickness behaviour or Neuroinflammation can be treated with Steroids like glucocorticoid, dexamethasone, Non steroidal anti-inflammatory drugs (NSAIDs) like selective COX 1 inhibitor (Resveratrol), selective COX 2 inhibitor (Celecoxib, Rofecoxib & Etoricoxib) & Non selective COX inhibitors (Aspirin, Ibuprofen) and a number of proteins produced by rDNA technology. Based on this, the present study was focused to analyse the protective effect and antioxidant studies of COX inhibitors.

MATERIALS AND METHODS

Animals

Wistar albino animals (200-250 gms) bred in central animal house of C. L. Baid Metha College of pharmacy was used. The animals were housed under standard conditions of light and dark cycle with free access to food and water. The experimental protocols were approved by Institutuional Animal Ethical Committee. (Approval no: IAEC/XXXIII/06/CLBMCP/2011 DATED: 27/09/2011)

Drugs

Lipopolysaccharide and Resveratrol were dissolved in normal saline (0.9% NaCl and DMSO) and given at a dose of 0.5 ml by i. p. injection while Celecoxib and Aspirin were prepared in normal saline and given at a dose of 0.5 ml by p. o. route. The drug solutions were prepared freshly at the beginning of each experiment.

Experimental protocol

Male albino wistar rats were divided into 8 groups and each group consisting of 6 rats. The drug treatment has done for one week according to the plan of study.

Group I:-Normal control treated with saline (0.5 ml-1 ml i. p)

Group II:-Negative control; saline for 6 days and LPS on 7th day followed by saline administration.

Group III:-Pre-treatment with Selective COX-1 inhibitor, Resveratrol (50 mg/kg p. o) for 6 days and LPS on 7th day followed by Resveratrol administration.

Group IV:-Pre-treatment with Selective COX-1 inhibitor, Resveratrol (100 mg/kg p. o) for 6 days and LPS on 7th day followed by Resveratrol administration.

Group V:-Pre-treatment with Selective COX-2 inhibitor, Celecoxib (10 mg/kg p. o) for 6 days and LPS on 7th day followed by Celecoxib administration.

Group VI:-Pre-treatment with Selective COX-2 inhibitor, Celecoxib (50 mg/kg p. o) for 6 days and LPS on 7th day followed by Celecoxib administration.

Group VII:-Pre-treatment with Non Selective COX inhibitor, Aspirin (100 mg/kg p. o) for 6 days and LPS on 7th day followed by Aspirin administration.

Group VIII:-Pre-treatment with Non Selective COX inhibitor, Aspirin (200 mg/kg p. o) for 6 days and LPS on 7th day followed by Aspirin administration.

Before administration of the drug, body temperature [10] and rectal temperature[11] were recorded. After 2 hours of drug administration, body temperature, rectal temperature and behavioural testing were performed. After analyzing behavioural parameters, the animals were sacrificed by cervical dislocation in order to perform *in vitro* studies. The animals were sacrificed by cervical dislocation in order to perform *in vitro* studies. Rat brain was removed and rinsed with normal saline and the homogenate prepared in 10%(w/v) cold phosphate buffered saline (0.1 mol/l, pH 7.4) with the help of homogenizer were used for the study.

Assessment of locomotor behaviour [12]

The locomotor activity can be easily studied with the help of actophotometer, for this albino rats were divided into eight groups, each group comprising of six animals. The cytophotometer consists of a square arena (30 × 30 × 25 cm) with the 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.

Assessment of behavioural parameters

Hole board test [13]

Exploratory behaviour was evaluated in an open-field paradigm. The open field was made up of plywood and comprises of 40 X 50 X 60 cm dimensions. The entire apparatus was painted black and was divided into 16 squares with white lines on the floor. Each animal was placed at one corner of the apparatus and for the next 5 minutes they were observed for their ambulation such as line crossings and head dipping.

Forced swim test [13]

Male Albino rats weighing 200-250 g are used. They are brought to the laboratory at least one day before the experiment and are housed separately in Makrolon cages with free access to food and water. Rats are individually forced to swim inside a vertical Plexiglas cylinder (height: 40 cm; diameter: 18 cm, containing 15 cm of water maintained at 25 °C). Rats placed in the cylinders for the first time are initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2–3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. After 5–6 min immobility reaches a plateau where the rats remain immobile for approximately 80% of the time. After 15 min in the water, the rats are removed and allowed to dry in a heated enclosure (32 °C) before being returned to their home cages. They are again placed in the cylinder 24 h later and the total duration of immobility is measured during a 5 min test. Floating behaviour during this 5 min period has been found to be reproducible in different groups of rats. An animal is judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. Each animal was placed water and for the next 5 minutes they were observed for floating time.

Assessment of anti oxidant enzymes

Estimation of superoxide dismutase (SOD) [14]

The SOD activity in supernatant was measured by the method of Misra and Fridovich. The supernatant (500µl) was added to 0.800 ml of carbonate buffer (100 mM, pH 10.2) and 100µl of epinephrine (3 mM). The change in absorbance of each sample was then recorded at 480 nm in spectrophotometer for 2 min at an intervals of 15 sec. Parallel blank and standard were run for determination SOD activity. One unit of SOD is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto oxidation.

Estimation of catalase (CAT) [15]

Catalase activity was measured by the method of Aebi. 0.1 ml of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H2O2. The rate of decomposition of H2O2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as units/mg protein. A unit is defined as the velocity constant per second.

Estimation of lipid peroxidase (LPO) [16]

The level of Lipid peroxides was estimated by Thiobarbituric acid reaction method described by Ohkawa *et al.*212. To 0.2 ml of test sample, 0.2 ml of SDS, 1.5 ml of acetic acid and 1.5 ml of TBA were added. The mixture was made up to 4 ml with water and then heated in a water bath at 95 °C for 60 minutes. After cooling, 1 ml of water and 5 ml of n-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, the organic layer was taken and its absorbance was read at 532 nm. The level of lipid peroxides was expressed as n moles of MDA released/g wet tissue.

Estimation of glutathione reductase (GRD) [17]

Glutathione reductase was assayed by the method of Stahl *et al.* The reaction mixture containing 1 ml phosphate buffer, 0.5 ml EDTA, 0.5 ml GSSG and 0.2 ml of NADPH was made up to 3 ml with distilled water. After the addition of 0.1 ml of tissue homogenate, the change in optical density at 340 nm was monitored for 2 minutes at 30 seconds interval. One unit of the enzyme activity was expressed as n moles of NADPH oxidized/min/mg protein.

Estimation of reactive nitrite species [18]

Nitrite/nitrate species concentration was estimated in the brain by using Griess reagent. 100µl of sample was mixed with 100µl of freshly prepared Griess reagent (mixture of 0.1% N-1-napthyl-ethylenediamine in water and 1% sulphanilamide in 5% phosphoric acid) and absorbance was observed at 540 nm using Biorad ELISA reader. The levels were expressed as µg/ml of plasma.

Statistical analysis

The statistical analysis was carried by one way ANNOVA followed by Dunnet´s 't'test. P values $p<0.001$ was considered statistically significant using software Graph Pad Prism 5.

RESULTS

Effect of resveratrol, celecoxib and aspirin on body weight

There was a significant ($p<0.001$) decrease in body weight produced by negative control group animals when compared with control groups. Treatment with Celecoxib (50 mg/kg p. o.) and aspirin (200 mg/kg p. o.) does not show any significant difference between before and after treatment. Results are given in table 1

Effect of resveratrol, celecoxib and aspirin on rectal temperature

Rectal temperature in negative control group animals were increased significantly ($p<0.001$) on comparison with control group animals. Treatment with Celecoxib (10 and 50 mg/kg p. o) and Aspirin (100 and 200 mg/kg p. o) showed significant ($p<0.01$) decrease in rectal temperature on comparison between before and after treatment. Results are given in table 2

Effect of resveratrol, celecoxib and aspirin on locomotor activity

There was a significant ($p<0.001$) decrease in the activity scores produced by Negative control group animals when compared with control group animals. Treatment with Celecoxib (50 mg/kg p. o.) and Aspirin (100 and 200 mg/kg p. o.) showed significant ($p<0.001$, $p<0.05$ and $p<0.001$) increase in the activity scores on comparison with Negative control group animals. Results are given in table 3.

Effect of resveratrol, celecoxib and aspirin in hole board model

The negative control group animals exhibited decreased line crossings and head dippings on comparison with control group animals ($p<0.001$). Treatment with Celecoxib (10 and 50 mg/kg p. o)

and Aspirin (200 mg/kg p. o) showed significant (p<0.05, p<0.01 and p<0.001) difference, in head dipping behaviour on comparison with negative control group animals. There was no significant difference in head dipping behaviour between negative group animals and Resveratrol (50 and 100 mg/kg p. o.) treated animals. Treatment with Resveratrol (100 mg/kg p. o.), Celecoxib (10 and 50 mg/kg p. o.) and Aspirin (100 and 200 mg/kg p. o.) showed significant (p<0.001, p<0.01, p<0.001, p<0.001 and p<0.001) increase in line crossing behavior on comparison with negative group animals. Results are given in table 4.

Effect of resveratrol, celecoxib and aspirin in forced swimming test

There was significant (p<0.001) increase in floating time in negative control group animals on comparison with control group animals. Treatment with Resveratrol (50 and 100 mg/kg p. o.) and Celecoxib (10 mg/kg p. o.) does not show any significant difference in floating time when compared with negative group animals. There was significant (p<0.001) decrease in floating time with Celecoxib, (50 mg/kg p. o) and Aspirin (100 and 200 mg/kg p. o) treated animals on comparison with negative group animals. Results are given in table 5

Effect of resveratrol, celecoxib and aspirin on superoxide dismutase (sod) levels

The negative control group animals showed significant (p<0.001) decrease in SOD level when compared to control group animals. The Celecoxib (10 and 50 mg/kg) treated animals did not show any significant increase while Resveratrol (50 and 100 mg/kg) showed significant (p<0.01 and p<0.001) increase when compared to negative group animals. Aspirin (100 and 200 mg/kg) treated animals showed significant (p<0.001) increase in brain SOD level. Results are given in Table-6 and fig 6.

Effect of drugs resveratrol, celecoxib and aspirin on catalase

The negative control group animals showed significant (p<0.001) decrease in catalase level when compared to control group animals. The Celecoxib (10 mg/kg) treated animals did not show any significant increase while Celecoxib (50 mg/kg), Resveratrol (50 and 100 mg/kg) showed significant (p<0.01, p<0.01 and p<0.001) increased brain catalase levels when compared to negative group animals. Aspirin (100 and 200 mg/kg) treated animals showed significant (p<0.001)increase in brain Catalase level. Results are given in table 7

Effect of drugs resveratrol, celecoxib and aspirin on lipid peroxide level

The negative control group animals showed significant (p<0.001) decrease in LPO level when compared to control group animals. The Celecoxib (10 mg/kg) treated animals did not show any significant increase while Celecoxib (50 mg/kg), Resveratrol (50 and 100 mg/kg) showed significant (p<0.01, p<0.05 and p<0.001) increased brain catalase levels when compared to negative group animals. Aspirin (100 and 200 mg/kg) treated animals showed significant (p<0.001) increase in brain LPO level. Results are given in table 8

Effect of drugs resveratrol, celecoxib and aspirin on glutathione reductase

The negative control group animals showed significant (p<0.001) decrease in GRD level when compared to control group animals. The Celecoxib (10 and 50 mg/kg) and Resveratrol (50 mg/kg) treated animals did not show any significant increase while Resveratrol (100 mg/kg) showed significant (p<0.01) increased GRD levels when compared to negative group animals. Aspirin (100 and 200 mg/kg) treated animals showed significant (p<0.01 and p<0.001) increase in brain GRD level. Results are given in table 9

Effect of drugs resveratrol, celecoxib and aspirin on nox levels

The negative control group animals showed significant (p<0.001) decrease in NOx level when compared to control group animals. The Celecoxib (10 mg/kg) and Resveratrol (50 mg/kg) treated animals did not show any significant increase while Celecoxib (50 mg/kg) and Resveratrol (100 mg/kg) showed significant (p<0.05 and p<0.01) increased NOx levels when compared to negative group animals. Aspirin (100 and 200 mg/kg) treated animals showed significant (p<0.01 and p<0.001) increase in brain GRD level. Results are given in table 10.

Table 1: Effect of drugs (Resveratrol, Celecoxib, and Aspirin) on body weight

Groups	Treatment groups	Body weight	
		Before treatment	After treatment
I	Control	146.66±3.33	148.00±3.88
II	Negative Control	197.50±6.02	186.66±7.49
III	Resveratrol (50 mg/kg p. o.)	177.50±5.12	170.83±3.27
IV	Resveratrol (100 mg/kg p. o.)	172.50±5.73	172.83±5.9
V	Celecoxib (10 mg/kg p. o.)	220.83±6.37	221.00±6.26
VI	Celecoxib (50 mg/kg p. o.)	197.50±3.81	197.83±3.37
VII	Aspirin (100 mg/kg p. o.)	204.16±3.96	206.66±4.94
VIII	Aspirin (200 mg/kg p. o.)	195.00±4.47	199.16±6.50

The values were expressed as mean±SEM of 6 animals, Comparison were made between before and after treatment, Statistical significant test for comparison was done by Two way Annova

Table 2: Effect of drugs (Resveratrol, Celecoxib and Aspirin) on rectal temperature

Groups	Treatment groups	Rectal temperature (°C)	
		Before treatment	After treatment
I	Control	36.51±0.16	36.45±0.15
II	Negative Control	36.78±0.10	38.26±0.18
III	Resveratrol (50 mg/kg p. o.)	36.41±0.21	36.66±0.14ns
Iv	Resveratrol (100 mg/kg p. o.)	36.90±0.14	36.96±0.19ns
V	Celecoxib (10 mg/kg p. o.)	37.16±0.13	37.13±0.16**
VI	Celecoxib (50 mg/kg p. o.)	37.05±0.14	36.98±0.17**
VII	Aspirin (100 mg/kg p. o.)	37.06±0.12	37.07±0.14**
VIII	Aspirin (200 mg/kg p. o.)	37.33±0.11	37.15±0.08**

The values were expressed as mean±SEM of 6 animals, Comparison were made between before and after treatment, Statistical significant test for comparison was done by One way Annova, followed by Dunnet´s 't'test test, *p<0.05, **p<0.01, ***p<0.001.

Table 3: Effect of drugs (Resveratrol, Celecoxib and Aspirin) on locomotor activity

Groups	Treatment groups	Locomotor activity in scores
I	Control	320.66±13.60
II	Negative Control	127.00±11.53
III	Resveratrol (50 mg/kg p. o.)	126.50±9.95
IV	Resveratrol (100 mg/kg p. o.)	126.16±8.60
V	Celecoxib (10 mg/kg p. o.)	167.16±15.38
VI	Celecoxib (50 mg/kg p. o.)	219±18.60***
VII	Aspirin (100 mg/kg p. o.)	190.00±14.36*
VIII	Aspirin (200 mg/kg p. o.)	280.33±15.18***

The values were expressed as mean±SEM of 6 animals, Comparison were made between Group I with Group II and Group II with III, IV,V,VI,VII,VIII, Statistical significant test for comparison was done by One way Annova, followed by Dunnet´s 't'test, *p<0.05, **p<0.01, ***p<0.001.

Table 4: Effect of drugs (Resveratrol, Celecoxib, and Aspirin) on hole board model

Groups	Treatment groups	Line crossings	Head dippings
I	Control	50.83±0.98	6.50±0.67
II	Negative Control	19.00±2.26***	1.33±0.49
III	Resveratrol (50 mg/kg p. o.)	23.16±1.49ns	1.33±0.42ns
IV	Resveratrol (100 mg/kg p. o.)	33.16±2.08***	1.66±0.42ns
V	Celecoxib (10 mg/kg p. o.)	29.16±1.99**	3.66±0.49*
VI	Celecoxib (50 mg/kg p. o.)	39.66±2.24***	4.16±0.54**
VII	Aspirin (100 mg/kg p. o.)	41.16±1.88***	3.16±0.30ns
VIII	Aspirin (200 mg/kg p. o.)	47.33±2.20***	5.33±0.49***

The values were expressed as mean±SEM of 6 animals, Comparison were made between Group I with Group II and Group II with III, IV,V,VI,VII,VIII, Statistical significant test for comparison was done by One way Annova, followed by Dunnet´s 't'test, *p<0.05, **p<0.01, ***p<0.001

Table 5: Effect of drugs (Resveratrol, Celecoxib and Aspirin) on forced swimming

Groups	Treatment groups	Floating time in secs
I	Control	22.33±2.15
II	Negative Control	142.16±4.42
III	Resveratrol (50 mg/kg p. o.)	139.00±4.35ns
IV	Resveratrol (100 mg/kg p. o.)	135±3.84ns
V	Celecoxib (10 mg/kg p. o.)	127.00±4.05ns
VI	Celecoxib (50 mg/kg p. o.)	101.00±3.59***
VII	Aspirin (100 mg/kg p. o.)	59.66±9.35***
VIII	Aspirin (200 mg/kg p. o.)	38.16±4.35***

The values were expressed as mean±SEM of 6 animals, Comparison were made between Group I with Group II and Group II with III, IV,V,VI,VII,VIII, Statistical significant test for comparison was done by One way Annova, followed by Dunnet´s 't'test, *p<0.05, **p<0.01, ***p<0.001.

Table 6: Effect of drugs (Resveratrol, Celecoxib and Aspirin) on superoxide dismutase (SOD) levels

Groups	Treatment groups	SOD (Units/mg protein)
I	Control	5.99±0.12
II	Negative Control	3.06±0.05
III	Resveratrol (50 mg/kg p. o.)	3.77±0.16**
IV	Resveratrol (100 mg/kg p. o.)	5.42±0.13***
V	Celecoxib (10 mg/kg p. o.)	2.85±0.03ns
VI	Celecoxib (50 mg/kg p. o.)	3.29±0.05ns
VII	Aspirin (100 mg/kg p. o.)	5.54±0.16***
VIII	Aspirin (200 mg/kg p. o.)	6.36±0.17***

The values were expressed as mean±SEM of 6 animals, Comparisons were made between: Group II with Group III, IV,V, VI, VII and VIII , Statistical significant test for comparison was done by one way ANNOVA, followed by Dunnet´s 't'test, ns-non significant, *p<0.05, **p<0.01, ***p<0.001.

Table 7: Effect of drugs (Resveratrol, Celecoxib and Aspirin) on catalase

Groups	Treatment groups	Catalase (Units/mg protein)
I	Control	5.43±0.23
II	Negative Control	2.78±0.02
III	Resveratrol (50 mg/kg p. o.)	4.33±0.10**
IV	Resveratrol (100 mg/kg p. o.)	4.73±0.09***
V	Celecoxib (10 mg/kg p. o.)	3.08±0.02ns
VI	Celecoxib (50 mg/kg p. o.)	3.46±0.05**
VII	Aspirin (100 mg/kg p. o.)	4.22±0.04***
VIII	Aspirin (200 mg/kg p. o.)	5.82±0.04***

The values were expressed as mean±SEM of 6 animals, Comparisons were made between: Group II with Group III, IV,V, VI, VII and VIII, Statistical significant test for comparison was done by one way ANNOVA, followed by Dunnet´s 't'test, ns-non significant, *p<0.05, **p<0.01, ***p<0.001.

Table 8: Effect of drugs (Resveratrol, Celecoxib and Aspirin) on lipid peroxides

Groups	Treatment groups	LPO(units/mg tissue)
I	Control	137.45±3.07
II	Negative Control	89.78±1.08
III	Resveratrol (50 mg/kg p. o.)	98.29±0.56*
IV	Resveratrol (100 mg/kg p. o.)	105.72±1.45***
V	Celecoxib (10 mg/kg p. o.)	95.42±1.30ns
VI	Celecoxib (50 mg/kg p. o.)	100.82±2.40**
VII	Aspirin (100 mg/kg p. o.)	106.73±3.42***
VIII	Aspirin (200 mg/kg p. o.)	132.70±1.98***

The values were expressed as mean±SEM of 6 animals, Comparisons were made between: Group II with Group III, IV,V, VI, VII and VIII, Statistical significant test for comparison was done by one way ANNOVA, followed by, Dunnet´s 't'test, ns-non significant, *p<0.05, **p<0.01, ***p<0.001.

Table 9: Effect of drugs (Resveratrol, Celecoxib and Aspirin) on glutathione reductase

Groups	Treatment groups	GRD(units/mg tissue)
I	Control	2.70±0.66
II	Negative Control	1.22±0.03
III	Resveratrol (50 mg/kg p. o.)	1.32±0.025ns
VI	Resveratrol (100 mg/kg p. o.)	1.75±0.05**
V	Celecoxib (10 mg/kg p. o.)	1.17±0.04ns
VI	Celecoxib (50 mg/kg p. o.)	1.33±0.04ns
VII	Aspirin (100 mg/kg p. o.)	2.26±0.10**
VIII	Aspirin (200 mg/kg p. o.)	2.56±0.10***

The values were expressed as mean±SEM of 6 animals, Comparisons were made between: Group II with Group III, IV,V, VI, VII and VIII, Statistical significant test for comparison was done by one way ANNOVA, followed by Dunnet´s 't'test, ns-non significant, *p<0.05, **p<0.01, ***p<0.001.

Table 10: Effect of drugs (Resveratrol, Celecoxib and Aspirin) on NOx

Groups	Treatment groups	NOx (units/mg tissue)
I	Control	0.61±0.1
II	Negative Control	1.82±0.01
III	Resveratrol (50 mg/kg p. o.)	1.71±0.02*
IV	Resveratrol (100 mg/kg p. o.)	1.69±0.03**
V	Celecoxib (10 mg/kg p. o.)	1.76±0.01ns
VI	Celecoxib (50 mg/kg p. o.)	1.71±0.03*
VII	Aspirin (100 mg/kg p. o.)	0.75±0.01**
VIII	Aspirin (200 mg/kg p. o.)	0.50±0.01***

The values were expressed as mean±SEM of 6 animals, Comparisons were made between: Group II with Group III, IV,V, VI, VII and VIII, Statistical significant test for comparison was done by one way ANNOVA, followed by Dunnet´s 't'test, ns-non significant, *p<0.05, **p<0.01, ***p<0.001.

DISCUSSION

Lipopolysaccharide, the principle components of all gram negative bacteria have been extensively studied as a major factor contributing to the pathogenesis of bacterial infections. LPS induces the production and release of inflammatory cytokines (IL-1, IL-6 and TNF-α) and COX enzymes of which several reactive oxygen species (ROS) are produced from cells (neutrophils, macrophages and other phagocytic cells) creating oxidative stress [19]. The inflammatory mediators relay signal to the CNS macrophages and microglia to produce the same cytokines, targeting neuronal substrates and eliciting sickness behaviour. Sickness behaviour is characterized by non-specific symptoms such as fever, prolong sleep, decrease in food and water intake, reduced mobility, depression and anxiety.

Drugs that are useful in the treatment of LPS induced sickness behaviour includes Selective COX-1 inhibitors such as Resveratrol, SC-560, Selective COX-2 inhibitors such as Celecoxib,NS-398, Non Selective COX inhibitors such as Aspirin, Indomethacin, piroxicam etc., and Glucocorticoids such as dexamethasone. Aspirin is mostly used drug to treat neuroinflammation, as it inhibits predominantly COX-2 enzyme. Because of its ulcer inducing property, we moved to Resveratrol and Celecoxib to perform our study.

In the present study, it was shown that the decrease in body weight and increase in rectal temperature was seen with the negative control group. Celecoxib treated group showed no changes in body weight before and after treatment but it showed the decrease in rectal temperature when compared to the negative control group and between before and after treatment.

Locomotor activity was found to be decreased in negative control groups when compared with control group animals. Celecoxib (50 mg/kg p. o) and Aspirin (100 and 200 mg/kg p. o) treated animals showed increase in locomotor activity when compared to Negative control group. Resveratrol did not show any difference in locomotor activity on comparing with negative control groups.

The hole board test provides a simple method for measuring the response of an animal to an unfamiliar environment and is widely used to measure anxiety. It was shown that social exploratory behaviour such as head dipping and line crossing behaviour have been enhanced. In the present study Celecoxib at 10 and 50 mg/kg p. o. showed increase in both line crossings and head dipping whereas Resveratrol at 100 mg/kg showed increase in line crossings.

Forced swimming test is a novel test to identify the depressant activity by measuring the floating time. In the present study, Celecoxib at dose of 50 mg/kg showed decrease in floating time when compared to negative control groups. Floating time have been increased in negative group animals when compared to control group animals.

Antioxidants are being investigated for the ability to prevent cardiovascular, hepatic and pulmonary damage produced by LPS induced production of ROS, peroxides and cytokines. Because

cytokines are behaviourally active, we hypothesize that antioxidants would inhibit LPS induced sickness behaviour. In our study, it revealed that Resveratrol shown to be effective against LPS induced behavioral alterations. Recent studies show treatment with antioxidants reduces ROS and TNF-α, IL-1β and IL-6 in LPS stimulated macrophages [20]. Resveratrol at doses 50 and 100 mg/kg p. o. showed a significant increase in Superoxide dismutase, Catalase, Glutathione reductase levels but significantly decreases Lipid peroxidation level, NOx when compared to control animals.

CONFLICT OF INTERESTS

Declared None

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