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Protective Effect of Resveratrol and Celecoxib on Lipopolysaccharide induced Oxidative Stress

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Abstract

Aim: We investigated the protective effect of selective COX-1 (resveratrol) and COX-2 (celecoxib) inhibitors on Lipopolysaccharide (LPS) induced oxidative damage in brain homogenates on different region such as cortex, straitum and hippoacampus.

Methods: Rats were injected with a single dose of LPS 100 µg/kg with or without pretreatment of resveratrol or celecoxib for seven days. Sixteen hours later, rats were sacrificed and brain were collected for antioxidant estimation such as Lipid peroxidation, reduced glutathione, superoxide dismutase and nitrites.

Results: Pretreated with resveratrol significantly reduced the level of TBARS, but not in celecoxib treated groups compared to LPS treated groups. Both the selective COX 1 and 2 inhibitors restoring the reduced glutathione level in all the regions and significantly reduced the superoxide dismutase in some regions, levels of nitrites were normalized only in selective COX 1 inhibitor but not in selective COX 2 inhibitor when compared with LPS treated rats.

Conclusion: From our results of the present study suggest that COX inhibitors are protected against LPS induced oxidative stress in rat brain tissue homogenate. Amongst the COX inhibitors, resveratrol possess more antioxidant property than celecoxib which states resveratrol having polyphenolic compound is considered to be potent antioxidant property than celecoxib on LPS induced oxidative damage.

Keywords: Lipopolysaccharide, oxidative stress, celecoxib, resveratrol.

INTRODUCTION

Oxidative stress is considered as a state of imbalance between the generation and scavenging of reactive oxygen species resulting into excess free radicals and thus impairment of neuronal integrity [1]. Under physiological conditions free radicals are immediately deactivated by antioxidant enzymes, such as glutathione peroxidase, catalase and superoxide dismutase [2]. The risk of oxidative stress is increased by ageing, exposure to pesticides, various environmental toxins and during inflammation [3].

Lipopolysaccharide is a bacterial endotoxin is known to stimulate the immune system through activation of macrophages in peripheral tissues. LPS also activates glial cells in brain and astrocytes releasing proinflammatory cytokines such as interleukin (IL-1β) and tumor necrosis factor (TNF-α) [4]. Activation of microglia has been observed during the development of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases (5). A recent report reveals that activated microglia releases inflammatory cytokines such as IL-1β and TNF-α and also produces oxygen and nitrogen centered free radicals that contribute to the neurodegenerative processes (6). LPS are mediated through with a member of the toll like receptor (TLR-4). The TLR-4 upon activation by LPS triggers a signaling cascade that ultimately induces the transcription of inflammatory cytokines such as TNF-α and IL-6 via NF-κB and oxidative stress activation [7]. In response to these cytokines, several reactive oxygen species are produced, establishing a status of oxidative stress [8].

In many *in vivo* and *in vitro* animal models, a relationship between LPS administration and the resulting overproduction of reactive oxygen species, including free radicals such as superoxide anion ($O_{2\cdot}$) and hydroxyl radical (OH), has been demonstrated. In the very early stages of localized cellular responses to LPS, endothelial and smooth muscle cells release hydrogen peroxide (H_2O_2), which functions to enhance micro vascular blood flow to the site of infection. The later phase inflammatory response to LPS creates an abundance of peroxides and reactive oxygen species (ROS), primarily from macrophages and infiltrating neutrophils [9]. Nitrite/nitrate possessing properties of a free radical, is an important molecule involved in the inflammatory response, is a reactive nitrogen species (RNS). The main sources of ROS in inflammatory process are both damaged mitochondria and activated microglia [10].

Cyclooxygenases (COXs) enzyme converts arachidonic acid into prostaglandins and has been suggested to play an important role in various neurodegenerative disorders [11]. COX exists as two isoforms including COX-1 and COX-2 enzymes. COX-1 is constitutive and participates in normal physiology whereas COX-2 is inducible and its expression is reported to be increased in several neurological diseases including Alzheimer's disease [12]. Recent studies also have indicated a previously unrecognized proinflammatory role of COX-1 in the pathophysiology of acute and chronic neurological disorders (10). Resveratrol [3, 4', 5 trihydroxystilbene] is a naturally occurring phytoalexin present in high concentration in skin of grapes and wine. It is believed to afford strong cardio-protective, anti-inflammatory and neuroprotective properties of red wine intake [13]. Celecoxib exhibited a number of potentially useful clinical effects such as cerebral stroke, AIDS, dementia and Parkinson's disease [14]. In addition, neuroprotective effects of COX-2 inhibitors have also been demonstrated in various CNS related disorders [15, 16]. Despite various studies that demonstrated the potential role of prostaglandins in the brain, there are no studies that addressed the potential effects of selective COX 1 inhibitors or selective COX-2 inhibitors on LPS induced oxidative damage in rat. With this background the main aim of present study was designed to examine the

effect of selective COX 1 (Resveratrol) and COX 2 inhibitor (Celecoxib) on LPS induced oxidative stress in rat.

MATERIALS AND METHODS

Animals

Adult Male Wistar rats (180-200g) were procured from C L Baid Metha College of Pharmacy, Thorapakkam, and Chennai and divided into six groups of six animals each. The rats were housed in colony cages at an ambient temperature of 25ºc ± 2ºc and 40-65% RH with a 12:12 h L:D cycle. The animals had free access to standard pellet diet and drinking water. The study was approved by institutional animal ethical committee (IAEC) and work was carried out as per CPCSEA guidelines, New Delhi.

Chemicals and reagents

Celecoxib (Madras pharmaceuticals Pvt Ltd. Chennai), Resveratrol, LPS; Serotype 055:B5 (Sigma Aldrich USA). All other chemicals and reagents unless specified were of analytical grade.

Experimental protocol and treatment schedule

To observe the effects of COX inhibitors on LPS induced oxidative damage in rat brain, 36 rats were randomly divided into 6 groups. The first group (control) received normal saline once daily for 7 days. The second group (LPS and saline) received normal saline once daily for 7 days followed by a single challenge of LPS 100µg/kg at day 7. Remaining groups III to VI administered COX inhibitors for 7 days followed by a single LPS 100µg/kg at day 7 after 30 min of COX inhibitors.

Groups	Treatment (mg/kg)
I.	Normal saline (i.p.)
II.	LPS 100 µg/kg (i.p.)
III	LPS 100 µg/kg (i.p.) + Resveratrol 10 mg/kg (p.o.)
IV	LPS 100 µg/kg (i.p.)+ Resveratrol 20 mg/kg (p.o.)
V.	LPS 100 µg/kg (i.p.) + Celecoxib 10 mg/kg (p.o.)
VI.	LPS 100 µg/kg (i.p.)+ Celecoxib 20 mg/kg (p.o.)

Tissue collection

Rats were perfused through heart with ice cold normal saline after 16 hours of the LPS injection under mild ether anesthesia. The brain was carefully removed and kept over a petri plate placed on ice for 15 min. Brain was dissected and isolated three different regions such as Striatum (STR), frontal cortex (FC), hippocampus (HP) [17].

Assay of reduced glutathione (GSH)

Tissue homogenate was added to an equal volume of ice cold 5% TCA. The precipitate was removed by centrifugation at 4000rpm for 10min. To 1ml aliquot of supernatant, 0.25ml of 0.2M phosphate buffer (pH 8.0) and 0.5ml of DTNB (0.6mM in 0.2M phosphate buffer, pH 8.0) were added and mixed well. The absorbance was read at 412nm using spectrophotometer (Perkin Elmer, 25, USA). The values were expressed in nanomoles/g tissue [18].

Assay of thiobarbituric acid reactive substances (TBARS)

Tissue homogenate was taken and to this 0.8ml saline, 0.5ml of BHT and 3.5ml TBA reagent (0.8%) were added and incubated at 60◦C in a boiling water bath. After cooling, the solution was centrifuged at 2000rpm for 10min.The absorbance of the supernatant was determined at 532nm using spectrophotometer (Perkin Elmer, 25, USA) against the blank [19].

Assay of superoxide dismutase (SOD)

Superoxide dismutase was assayed by taking 0.05ml of tissue homogenate followed by the addition of 0.3ml of sodium pyrophosphate buffer (0.025M, pH 8.3), 0.025ml of PMS (186M) and 0.075ml of NBT (300M in buffer,pH 8.3). Reaction was started by addition of 0.075ml of NADH (780M in buffer of pH 8.3). After incubation of the reaction mixture at 30◦C for 90s, the reaction was stopped by addition of glacial acetic acid (0.25ml). Then, the reaction mixture was stirred vigorously and shaken with 2.0 ml of n-butanol. The mixture was allowed to stand for 10min and centrifuged. 1.5ml of n-butanol alone served as blank. The colour intensity was read at 560nm using spectrophotometer (Perkin Elmer, 25, USA). Enzyme activity was expressed as 1 Unit=50% inhibition/minute/mg of protein [20].

Nitrite estimation

Nitrite was assayed by taking 0.2ml of 10% homogenate followed by addition of 1.8ml of saline and 0.4ml of 35% sulphosalicylic acid for protein precipitation. The precipitate was removed by centrifugation at 4000rpm for 10min. To 1ml aliquot of supernatant, 2ml Griess reagent (1g of sulphanilamide dissolved in small volume of water, 2ml of orthophosphoric acid and 100mg of naphthyl ethyldiamine were added. Volume was made up to 100ml with distilled water and mixed well). The mixture was allowed to stand for 20min under dark conditions. The colour intensity was read at 540nm (Perkin Elmer, 25, USA). Standard calibration was plotted using sodium nitrite in the concentration range 200–1000ng [21].

Statistical analysis

Data was expressed as mean ± S.E.M. Mean difference were analyzed by one way ANOVA followed by Tukey's Multiple Comparison test. Statistical analysis was performed using Graph Pad Prism, 5.01 (San Diego, US). $P<0.05$ was fixed as the statistical significance criterion.

RESULTS

Effect of COX inhibitors on LPS induced reduced glutathione (GSH) in different brain regions

In cortex, striatum and hippocampus brain regions, LPS 100µg/kg produced significantly ($p<0.05$, $p<0.001$ and $p<0.05$) decrease in GSH levels in comparison to control groups. Treatment with selective COX 1 inhibitor resveratrol 10mg/kg and 20mg/kg showed significantly ($p<0.001$, $p<0.01$, $p<0.001$, $p<0.001$, $p<0.001$ and $p<0.001$) increased in GSH level in all the regions compared with LPS treated rats. Celecoxib 10mg/kg showed significant ($p<0.001$) increased in GSH level only in cortex region when compared to LPS treated rats. Celecoxib 20mg/kg showed significant ($p<0.001$, $p<0.05$ and $p<0.001$) increased in GSH level in all the three brain regions when compared to LPS treated rats. (Table 1)

Table 1: The effect of COX inhibitors attenuated on LPS induced reduced glutathione (GSH) in different brain region

Group	Cortex	Striatum	Hippocampus
Control	34.24±0.5646	34.34±0.2569	31.40±0.4446
LPS100µg/kg	26.67±0.5876 ***	28.76±0.2012 ***	27.25±0.4154 ***
Resveratrol 10mg/kg	31.07±0.3011 ###	32.95±0.3702 ##	32.20±0.5267 ###
Resveratrol 20mg/kg	35.98±0.1689 ###	36.80±0.2975 ###	35.61±0.4594 ###
Celecoxib 10mg/kg	29.48±0.1901 ###	29.49±0.3321	28.38±0.5681
Celecoxib 20mg/kg	32.71±0.4029 ###	31.66±0.5657 #	33.46±0.4122 ###

Values are mean± SEM of three replicates (n=3). *** (p<0.001), ** (p<0.01) and *(p<0.05) compared to control rats. ### (p<0.001), ## (p<0.01) and # (p<0.05) compared to LPS treated rats.

Table 2: The effect of COX inhibitors attenuated on LPS induced Lipid Peroxidation (TBARS) in different brain regions

Group	Cortex	Striatum	Hippocampus
Control	1.46± 0.08	1.65± 0.01	1.58± 0.03
LPS100µg/kg	1.83± 0.02***	1.81± 0.02***	1.73± 0.08
Resveratrol 10 mg/kg	1.56± 0.04##	1.59± 0.02###	1.68± 0.03
Resveratrol 20 mg/kg	1.28± 0.04###	1.43± 0.01###	1.58± 0.04
Celecoxib 10 mg/kg	1.71± 0.04	1.82± 0.02	1.77± 0.02
Celecoxib 20 mg/kg	1.69± 0.04	1.72± 0.02	1.68± 0.02

Values are mean± SEM of three replicates (n=3). *** (p<0.001), ** (p<0.01) and *(p<0.05) compared to control rats. ### (p<0.001), ## (p<0.01) and # (p<0.05) compared to LPS treated rats

Table 3: The effect of COX inhibitors attenuated on LPS induced super oxide dismutase (SOD) in different brain region

Group	Cortex	Striatum	Hippocampus
Control	8.40±0.00	9.25±0.06	8.64±0.06
LPS100µg/kg	5.77±0.04***	5.91±0.18***	6.34±0.04
Resveratrol 10mg/kg	8.25±0.03###	9.06±0.05###	9.04±0.01
Resveratrol 20mg/kg	8.53±0.03###	9.15±0.14###	8.82±0.03
Celecoxib10mg/kg	6.87±0.04###	7.32±0.06###	6.70±0.05
Celecoxib 20mg/kg	6.54±0.03###	6.65±0.11##	7.04±0.04

Values are mean± SEM of three replicates (n=3). *** (p<0.001), ** (p<0.01) and *(p<0.05) compared to control rats. ### (p<0.001), ## (p<0.01) and # (p<0.05) compared to LPS treated rats

Table 4: The effect of COX inhibitors attenuated on LPS induced nitrites (NOx) in different brain region

Group	Cortex	Striatum	Hippocampus
Control	36.33±1.05	30.33±0.80	37.00±1.23
LPS100µg/kg	45.33±0.84***	37.33±0.66***	50.67±1.20***
Resveratrol 10mg/kg	39.00±1.52##	30.00±0.73###	45.50±1.70
Resveratrol 20mg/kg	35.00±1.34###	29.17±0.90###	43.33±1.52##
Celecoxib10mg/kg	43.33±0.84	38.33±0.61	49.00±0.73
Celecoxib 20mg/kg	42.17±1.04	35.83±1.10	50.17±1.10

Values are mean± SEM of three replicates (n=3). *** (p<0.001), ** (p<0.01) and *(p<0.05) compared to control rats. ### (p<0.001), ## (p<0.01) and # (p<0.05) compared to LPS treated rats

Effect of COX inhibitors on lipid peroxidation (TBARS) in different brain regions

Intraperitoneal administration of LPS 100µg/kg produced significant (p<0.01 and p<0.01) increase in TBARS level in cortex and striatum not in hippocampus as compared with respective control rats. Treatment with resveratrol 10mg/kg and 20mg/kg showed significant (p<0.01, p<0.001, p<0.001 and p<0.001) decrease in TBARS level in cortex and striatum but not in hippocampus region in both low as well as in high doses when compared with LPS treated rats. Treatment with celecoxib 10 & 20mg/kg did not alter the TBARS level significantly when compared with LPS treated rats (Table 2)

Effect of COX inhibitors on LPS induced super oxide dismutase

LPS 100 µg/kg treated rats significantly (p<0.001 and p<0.001) decreased the SOD level in cortex and striatum but not in hippocampus region as compared with their respective control. Treatment with Resveratrol 10mg/kg and 20mg/kg and Celecoxib 10mg/kg and 20mg/kg showed significant (p<0.001) increased in SOD level in cortex and striatum but not in hippocampus when compared with LPS treated rats. (Table 3)

Effect of COX inhibitors on LPS induced Nitrites

In all the brain regions, LPS 100µg/kg produced significant increase in nitrates level as compared with control rats ($p<0.001$). Treatment with resveratrol 10mg/kg showed significant ($p<0.01$ and $p<0.001$) decreases nitrites level in cortex and striatum not in hippocampus. Resveratrol 20mg/kg showed significant ($p<0.001$, $p<0.001$ and $p<0.01$) decreases in Nitrites level in all the brain regions. There was no significant difference produced in celecoxib treated rats in both low as well as high doses when compared with LPS treated rats. (Table 4)

DISCUSSION

We have characterized a rat model of LPS-induced acute neuroinflammation. This model leads to an acute inflammatory response in the brain regions linked with several neurodegenerative diseases accompanied by enhanced oxidative stress. Oxidative stress can cause cellular damage and neurodegeneration by inducing the reactive oxygen species (ROS) that oxidize vital cellular components such as lipids, proteins and DNA. The COX pathways are involved in ROS generation and responsible for neuronal cell death both *in vivo* and *in vitro* (22).

Brain region related neurotoxicity raises an important question whether vulnerability of neurons to free radical damage differ from one brain region to another. In our present study, we attempted to answer this question by observing the effect of oxidative stress inducers, LPS damages on different brain regions like cortex, striatum and hippocampus. Neurotoxins can generate free radical directly from itself or indirectly by activating microglia. LPS is an indirect inducer of oxidative stress generates ROS via the activation of microglia. In normal conditions, tissues have a functional anti-oxidative system that is depleted in oxidative stress. This study provides evidence on the presence of an acute neuroinflammation characterized by generation of oxidative damage such as LPO, SOD, GSH and nitrites following by LPS challenge. Glutathione is an endogenous antioxidant, which functions in tissue defense against oxidative stress induced by brain damage [23]. Brain mitochondria contain a large pool of GSH. Excessive generation of free radicals causes alteration in GSH level thus decrease in GSH concentration indicates oxidative stress. Lipid peroxidation results into the increase in the concentration of end product TBARS, Therefore, TBARS is used as an indicator of membrane lipid peroxidation and its elevated level is considered as marker of oxidative stress. Super oxide dismutase is an antioxidant defence in nearly all the cells exposed to oxygen. Nitrite/nitrate (NOx), possessing properties of a free radical, is an important molecule involved in the inflammatory response, is a reactive nitrogen species (RNS). Classically oxidative stress is described as an imbalance between generation and elimination of ROS and RNS. Thus effect of intracellular antioxidant marker and membrane damage in terms of GSH, MDA, SOD and NOx respectively was studied.

In the present study show that COX inhibitors attenuated LPS oxidative stress in rats, as indicated suppressed malondialdehyde concentration, through the elevation of antioxidative enzyme activities, such as SOD, GSH and nitrites. It is reflected by decrease in GSH in all brain regions following LPS injection is compatible with another similar study [24, 25]. The enhanced production of brain TBARS observed in our study by LPS injection is in agreement with the *in vivo* study [26] and *in vitro* [27]. Living organisms contain SOD, which removes superoxide, and are thus protected from injury caused by ROS. Our study revealed that LPS significantly suppressed SOD and nitrites activities in all the brain regions. The COX pathways are involved in ROS and RNS generation and responsible for neuronal cell death both *in vivo* and *in vitro* [26]. Therefore, inhibition of COX isoforms could prevent the LPS induced oxidative stress. Pretreatment with resveratrol 10 and 20 mg/kg and celecoxib 20mg/kg showed respective increase in the reduced glutathione level in all the three brain regions, celecoxib 10 mg/kg showed increased only in cortex region not in striatum and hippocampus. In TBARS level, pretreatment with resveratrol 10 and 20 mg/kg reversed the increase in lipid peroxidation in cortex and striatum not in hippocampus but celecoxib 10 and 20 mg/kg not reversed the lipid peroxidation in all the brain regions. Pretreatment with resveratrol 10 mg/kg and celecoxib 10 and 20 mg/kg increased SOD levels in cortex and striatum not in hippocampus. Decreased in nitrite levels in pretreatment with resveratrol 10mg/kg and 20mg/kg but not in celecoxib in low and higher doses. Previous reports supports our present study COX 1 deficient mice had a decreased oxidative stress after injection of LPS [27].

CONCLUSION

From our results of the present study suggest that COX inhibitors are protected against LPS induced oxidative stress in rat brain tissue homogenate. Amongst the COX inhibitors, resveratrol possess more antioxidant property than celecoxib which states resveratrol having polyphenolic compound is considered to be potent antioxidant property than celecoxib on LPS induced oxidative damage

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Simonian, N.A., Coyle, J.T., Oxidative stress in neurodegenerative diseases. Annu. Rev. Pharmacol. Toxicol. 1996, 36, 83–106.
2. Halliwell, B., Oxidative stress and neurodegeneration: where are we now?. J Neurochem. 2006, 97, 1634-1658.
3. Czapski, G.A., Czubowicz, K., Strosznajder R.P,. Evaluation of the antioxidative properties of lipoxygenase inhibitors. Pharmacol Rep. 2012, 64, 1179-1188.
3. Quan, N., Sundar S.K., Weiss J.M., Induction of interleukin-1 in various brain regions after peripheral and central injections of lipopolysaccharide. J. Neuroimmunol. 1994, 49, 125-134.
4. McGeer, P.L., Itagaki, S., Boyes, B.E., McGeer, E.G., Reactive microglia are positive for HLA-DR in the substantia nigra of

Parkinson's and Alzheimer's disease brains. Neurology. 1998, 38, 1285–1291.
5. Tanaka, S., Ide, M., Shibutani, T., Ohtaki, H., Numazawa, S., Shioda, S., Yoshida, T., Lipopolysaccharide-induced microglial activation induces learning and memory deficits without neuronal cell death in rats. J. Neurosci. Res. 2006, 83, 557–566.
4. Qin, L., Liu, Y., Wang, T., Wei, S. J., Block, M.L., Wilson, B., Liu, B., NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and proinflammatory gene expression in activated microglia. J Biol Chem. 2004, 279, 1415-1421.
5. Sugino, K., Dhi, K., Yamada, K., Kawasaki, T., The role of lipid peroxidation in endotoxin-induced hepatic damage and the protective effect of antioxidants. Surgery. 1987, 101, 746-752.
6. Khodr, B., Khalil, Z., Modulation of inflammation by reactive oxygen species: implications for aging and tissue repair. Free Radic Biol Med. 2001, 30, 1-8.
7. Sewerynek, E., Melchiorri, D., Chen, L., Reiter, R.J., Melatonin reduces both basal and bacterial lipopolysaccharide induced lipid peroxidation *in vitro.* Free Radic Biol Med. 1995, 19, 903-9.
8. Krakauer, T., Molecular therapeutic targets in inflammation: Cyclooxygenase and NF-kappaB. Curr. Drug Targets Inflamm Allergy. 2004, 3, 317-324.
9. Saldana, M., Pujols, L., Roca-Ferrer, J., Cardozo, A., Aguilar, E., Relevance of COX-2 gene expression in dementia with lewy bodies associated with Alzheimer pathology. Mov Disord. 2008, 23, 804–10.
10. Choi, S.H. et al., Genetic deletion or pharmacological inhibition of cyclooxygenase-1 attenuate lipopolysaccharide-induced inflammatory response and brain injury. FASEB J. 2008, 22, 1491–1501.
11. Pervai, S., Resveratrol: from grapevines to mammalian biology. FASEB J. 2003, 17, 1975-1985.
12. Candelario-Jalil, E., Gonzalez-Falcon, A., Garcia-Cabrera, M., Leon, O.S., Fiebich, B.L., Wide therapeutic time window for nimesulide neuroprotection in a model of transient focal cerebral ischemia in the rat. Brain Res. 2004, 1007, 98-108.
13. Asanuma, M., Miyazaki, I., Ogawa, N., Neuroprotective effects of nonsteroidal anti-inflammatory drugs on neurodegenerative diseases. Curr Pharm Des. 2004, 10, 695-700.
14. Galvao, R.I., Diogenes, J.P., Maia, G.C., Filho, E.A., Vasconcelos, S.M., de Menezes, D.B, et al., Tenoxicam exerts a neuroprotective action after cerebral ischemia in rats. Neurochem Res. 2005, 30:39-46.
15. Klivenyi, P., Kiaei, M., Gardian, G., Calingasan, N.Y., Beal, M.F., Additive neuroprotective effects of creatine and cyclooxygenase 2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis. J Neurochem. 2004, 88, 576-582.
16. Glowinski, J., Iversen, L.L., Regional studies of catecholamines in the rat brain-I. The disposition of [3H] norepinephrine, [3H] dopamine and [3H] dopa in various regions of the brain. J. Neurochem. 1966, 13, 655-70.
17. Jollow, D.J., Mitchell, J.R., Zampaglione, N., Gillete, J.R., Bromobenzene induced liver necrosis: Protective role of glutathione and evidence for 3, 4-bromobenzene as the hepatotoxic metabolite. Pharmacology. 1994, 11, 151-169.
18. Ohkawa, H., Ohishi, H., Yagi, K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Ann Biochem. 1979, 95, 351-358.
19. Kakkar, P., Das, B., Viswanathan, P.N., A modified spectrophotometric assay of superoxide dismutase. Indian J. Biochem. Biophy. 1984, 21, 130-132.
20. Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., Tannenbaum, S.R., Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal.Biochem. 1982, 126, 131-138.
21. Pepicelli, O., Fedele, E., Bonanno, G., Raiteri, M., Ajmone-Cat, M.A, Greco, A., *et al.,* In vivo activation of N-methyl-D-aspartate receptors in the rat hippocampus increases prostaglandin E (2) extracellular levels and triggers lipid peroxidation through cyclooxygenase-mediated mechanisms. J Neurochem. 2002, 81, 1028–34.
21. Jain, A., Maertensson, J., Stole, E., Auld, A.M., Meister, A., Glutathione deficiency leads to mitochondrial damage in brain. Proc. Natl. Acad. Sci. USA. 1991, 88, 1913-1917.
22. Cadenas, S., Cadenas, A.M., Fighting the stranger-antioxidant protection against endotoxin toxicity. Toxicology. 2002, 180, 145-63.
23. Mostafa, Y.H., Al-Shabanah, O.A., Hassan, M.T, Khairaldin, A.A., Al-Sawaf, H.A., Modulatory effects of N acetylcysteine and α-tocopherol on brain glutathione and lipid peroxides in experimental diabetic and endotoxin stressed rats. Saudi Pharm. J. 1994, 2, 64-9.
24. Sewerynek, E., Melchiorri, D., Chen, L., Reiter, R.J., Melatonin reduces both basal and bacterial lipopolysaccharide induced lipid peroxidation in vitro. Free Radic. Biol. Med. 1995, 19, 903-9.
25. Moghaddam, B., Stress preferentially increases extraneuronal levels of excitatory amino acids in the prefrontal cortex: comparison to hippocampus and basal ganglia. J. Neurochem. 1993, 60, 1650-7.
26. Pepicelli, O., Fedele, E., Bonanno, G., Raiteri, M., Ajmone-Cat, M.A., Greco, A., In vivo activation of N-methyl-D-aspartate receptors in the rat hippocampus increases prostaglandin E (2) extracellular levels and triggers lipid peroxidation through cyclooxygenases mediated mechanisms. J. Neurochem. 2002, 81, 1028-34.
27. Choi, S.H., Bosetti, F., Cyclooxygenase-1 null mice show reduced neuroinflammation in response to beta-amyloid. Aging (Albany, NY). 2009, 1, 234-244.