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Research Article

MODULATORY ROLE OF NIMESULIDE, CAFFEIC ACID AND THEIR COMBINATION AGAINST IMMUNOLOGICALLY INDUCED MOUSE MODEL OF CHRONIC FATIGUE SYNDROME

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ABSTRACT: Chronic Fatigue Syndrome (CFS) is a complex, debilitating illness characterized by persistent and relapsing fatigue that does not improve with rest. This study aimed to explore the neuroprotective effects of nimesulide, and caffeic acid and their combination as an antioxidant in the immunologically induced chronic fatigue-like condition. The CFS was assessed by water-immersion stress test and stress-induced hyperalgesia. Nimesulide (5 and 10 mg/kg), caffeic acid (5 and 10 mg/kg), and their low-dose combination (nimesulide 5mg/kg and caffeic acid 5mg/kg) were administered daily for 21 days. On the 22nd day, the brain of animals was isolated immediately after the behavioral assessments for estimation of oxidative stress markers (SOD, GSH, MDA, and nitrite). The mice challenged with lipopolysaccharide (LPS) used as immunogen (control group) followed by water immersion stress for 21 days showed a significant increase in immobility time and hyperalgesia. The rats also showed decreased levels of antioxidant defense enzymes (SOD, GSH, and MDA), and cortisol levels but markedly increased tumor necrosis factor-alpha (TNF-∝) levels. The daily drugs treated groups showed a significant ($p<0.05$) reduction in immobility time in stress-induced models and reversed various biochemical alterations as well as TNF-∝ and cortisol levels when compared to the control group. Furthermore, the lower dose combination of nimesulide and caffeic acid significantly ($p<0.05$) improved behavior performance and attenuated the chronic fatigue-like condition as compared to single drug-treated groups. The result of the present findings strongly demonstrates that the potential antioxidant effect of nimesulide and caffeic acid and its combination has protective effects against immunologic-induced fatigue and could be used in the management of chronic fatigue syndrome.

Key words: Chronic fatigue syndrome, Tumor necrosis factor, Nimesulide, Caffeic acid.

INTRODUCTION

Chronic fatigue syndrome (CFS) is a heterogeneous disorder of unknown etiology defined by persistent or relapsing debilitating mental and physical fatigue, which is exacerbated by minor exertion. (Cortes *et al*. 2019). Patients of CSF also experience further somatic symptoms, such as headache, myalgia, arthralgia, tender lymph nodes, cognitive disturbances, gastrointestinal (GI) disturbance, low-grade fever, visual disturbances, and paresthesia (Rowe *et al.* 2017).

Various neuroendocrine abnormalities also occur in CFS, which contribute to impaired energy and mood.

The diagnosis and treatment of chronic fatigue syndrome are also challenging because multiple systems including hormonal, neurologic, and immunologic are involved in CFS etiology (Lorusso and Ricevuti 2022). Stress and infection have been considered major contributors to the development of chronic fatigue syndrome. An alteration in cytokine profile, a decreased function of natural killer (NK) cells, and a reduced response of T cells to mitogens and other specific antigens have been reported (Cliff *et al.* 2019). Further, an increase in proinflammatory cytokines and key inflammatory mediators such as NF-kB, COX-2, and iNOS have been seen in CFS patients (Morris and Maes 2014, Yang *et al.* 2021). The involvement of oxidative and nitrosative stress pathways in CFS has been replicated in various studies (Nacul *et al.* 2020). There is no definite diagnosis or treatment for this syndrome. Various drugs have been tried with limited success. Various experimental studies have demonstrated the potential role of antioxidants in the managing of chronic fatigue syndrome (Nipate and

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Tiwari 2020). Since oxidative stress and inflammation have been suggested in the pathophysiology of CFS, these sites could be exploited as a probable target for the therapeutic management of CFS (Morris *et al*. 2018). Therefore, the present study has focused on the possible antioxidants and anti-inflammatory agents in the disease progression of CFS.

Caffeic acid (3, 4-dihydroxycinnamic acid) is one of the natural phenolic compounds known for its antioxidant and anti-inflammatory properties. Caffeic acid is a scavenger of a number of reactive species, including DPPH, hydroxyl radicals, superoxide anions, and peroxynitrite (Adjimani and Asare 2015). Anti-inflammatory activity confirms by its inhibitory effect on the 5- Lipoxygenase (5-LOX) enzyme and transcription factor NF-kB (Silva *et al.* 2020). Nimesulide (4-nitro-2-phenoxyethanol sulphonamide) is another compound preferential cyclooxygenase (COX)-2 inhibitor that belongs to the sulphonamide class of NSAIDs and causes fewer gastrointestinal side effects. Previous studies have demonstrated the antioxidant effect of nimesulide in LPS-induced oxidative stress and MPTP-induced neurotoxicity (Gupta *et al.* 2010).

The interplay of stress and infection in the pathogenesis of CFS (Blomberg *et al.* 2018). Not only infection but physical and psychological stress can also result in immune dysregulation partly through alteration in the production of proinflammatory cytokines (Maydych 2019). Many CFS patients have consistently observed impaired immune responses (Mensah *et al.* 2017). CFS can be induced by a single intraperitoneal injection of bacterial antigen as described by various researchers (Sheng *et al.* 2011). Interaction of LPS with macrophages results in the generation of proinflammatory cytokines and reactive oxygen species (ROS) viz. hydrogen peroxide, superoxide anions, hydroxyl radicals, and singlet oxygen (Castaneda *et al.* 2017).

In the light of above evidence, the present study was conducted to evaluate the modulatory role of nimesulide (a preferential COX-2 inhibitor) and caffeic acid (natural antioxidant and 5-LOX inhibitor) and their combination in LPS-induced fatigue in a murine model of water immersion stress and stress-induced hyperplasia.

MATERIALS AND METHODS

Experimental animals

Albino BALB/c mice (18-22g) purchased from the Institute of Microbial Technology, Chandigarh, India used for the study. The animals had free access to standard rodent food pellets and water. They were acclimatized to the laboratory conditions one day before the experiment and daily at least for 1 hr. before the experiment. All the experiments were conducted between 07:00 am to 12:00 pm. The experimental protocol was approved by the Institutional Animal Ethics Committee (MMCP/IEC/10/40) and conducted according to the National Science Academy Guidelines for the use and care of animals.

Experimental drugs

Lipopolysaccharide (LPS) from *E. coli* serotype 0111:B4 containing not less than 500,000 EU/mg (Sigma, St. Louis, USA), nimesulide (Pubmed CID-4495) (Panacea Biotec Ltd, India), caffeic acid (Pubmed CID-689043) and sodium carboxymethyl cellulose (Pubmed CID-24748) (Himedia, Mumbai, India). Animals were intraperitoneally injected with 1 mg/kg of LPS in distilled water. Different doses of nimesulide and caffeic acid were prepared in 0.25% w/v Na-CMC and were administered orally (p.o.) in a constant volume of 1 ml/100 gm.

Assessment of fatigue and its related paradigms water-immersion stress

The mice were forced to swim individually in glass jar (25×12×25 cm) containing water at room temperature (22±3 °C). The height of the water level was adjusted to 15 cm. After an initial period of vigorous activity, each animal assumed a typical immobile posture. The mice were considered to be immobile when they ceased to struggle and made minimal limb movements to keep their head above the water level. The immobility period was noted for 6 min in a total period of 10 min (Gupta *et al.* 2009, Vij *et al.* 2009).

Stress-induced hyperalgesia

Stress-induced hyperalgesia was assessed by the tail-immersion test. Mice were held individually and their tails were immersed in hot water (52.5±0.5 °C) for not more than 10 s as cut-off time and withdrawal latency were observed on the same days as the immobility was observed (Gupta *et al.* 2009, Vij *et al.* 2009).

Experimental design

Nine groups were employed in the present study, comprising six animals in each group. Group-I comprised the control group, where animals received an equivalent volume of vehicles for 21 days. Group II animals were challenged with a single intraperitoneal injection of LPS (1 mg/kg), followed by an equivalent volume of vehicle for 21 days. Group III and group-IV, included animals treated with nimesulide (10 mg/kg, p.o.) *per se* and caffeic acid (10 mg/kg, p.o.) *per se* respectively, for 21 days. Group V and group-VI, comprised animals that

received the LPS challenge as per Group II and were treated with nimesulide (5 mg/kg, p.o.) and (10 mg/kg, p.o.), respectively for 21 days. Group VII and group-VIII, comprised animals that received the LPS challenge as per Group II and were treated with caffeic acid (5 mg/kg, p.o.) and (10 mg/kg, p.o.), respectively for 21 days. Group IX, comprised animals that received the LPS challenge as per Group II and received a combination of nimesulide (5 mg/kg, p.o.) and caffeic acid (5 mg/kg, p.o.) for 21 days.

Drugs were administered 30 min before the LPS challenge followed by the recording of the immobility period and tail withdrawal latency. This was taken as the immobility time as well as hyperalgesia for day one. The drug treatment was given daily for 21 days consequently and animals were subjected to a water-immersion stress test for 10 min daily as well as an assessment of stress-induced hyperalgesia. The immobility duration and withdrawal latency was measured 30 min after drug administration on day 1st day, 7th day, 14th day and 21st day.

Estimation of Parameters

On the 22nd day of the study, the animals were sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture and serum was separated for the estimation of TNF-∝. The brains were immediately removed, raised in ice-cold saline, and weighed. A 10% w/v tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4) which was further used for the estimation of oxidative stress parameters.

Measurement of lipid peroxidation reduced glutathione and superoxide dismutase levels

The quantitative measurement of lipid peroxidation in the whole brain was done according to the method of Wills (1966). The amount of malondialdehyde (MDA), a measure of lipid peroxidation was assayed in the form of thiobarbituric acid reacting substances (TBARS). The results were calculated as nmol of MDA/mg protein and expressed as a percentage of the vehicle group.

Reduced glutathione in the brain was assayed by the method of Ellman (1959). The results were calculated as ì moles of GSH/mg protein and expressed as a percentage of the vehicle group. The determination of Superoxide Dismutase (SOD) levels in the brain was done by the method of Kono (1978). The results were calculated as units/mg protein and expressed as a percentage of the vehicle group.

Measurement of brain nitrite levels

The accumulation of nitrite in the whole brain, an indicator of the production of NO was determined with the colorimetric assay with Griess reagent (0.1 % N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green *et al.* (1982). The results were calculated as µg/ml and expressed as a percentage of the vehicle group.

Protein estimation

Protein determination is done by the Biuret method (Gornall *et al.* 1949). The amount of protein was calculated from a standard curve of Bovine serum Albumin.

Estimation of Tumor Necrosis Factor-alpha

Tumor necrosis factor-alpha (TNF-∝) was estimated by using a mouse TNF-∝ kit (R and D systems). It is a solid-phase sandwich enzyme-linked immune-sorbent assay (ELISA) using a microtitre plate reader at 450 nm. Concentrations of TNF-∝ were calculated from the plotted standard curves.

Statistical analysis

The results were expressed as mean ± SEM and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. In all the tests, the criterion to establish statistical significance was $p<0.05$. Statistical analysis was done using Graphpad Prism 5 software.

RESULTS AND DISCUSSION

Effect of nimesulide, caffeic acid and their combination on immobility time

LPS challenge significantly increased the immobility time as compared to the vehicle group ($p<0.05$) (Fig. 1). Chronic administration of nimesulide (5 and 10 mg/kg p.o.) or caffeic acid (5 and 10 mg/kg p.o.) for 21 days significantly decreased the immobility time in LPS-treated animals ($p<0.05$). Furthermore, the lower dose combination of nimesulide and caffeic acid (5 mg/kg p.o.) significantly ($p<0.05$) potentiated their protective effect in LPS-treated animals, which was also significant as compared to their effect by itself alone [$F(6,41)=30.09$, ($p<0.05$)] (Fig. 1).

Effect of nimesulide, caffeic acid and their combination on stress-induced hyperalgesia

Challenge with LPS significantly decreased the tail withdrawal latency in mice as compared to the vehicle group ($p<0.05$) (Fig. 2). Chronic administration of nimesulide (5 and 10 mg/kg p.o.) or caffeic acid (5 and

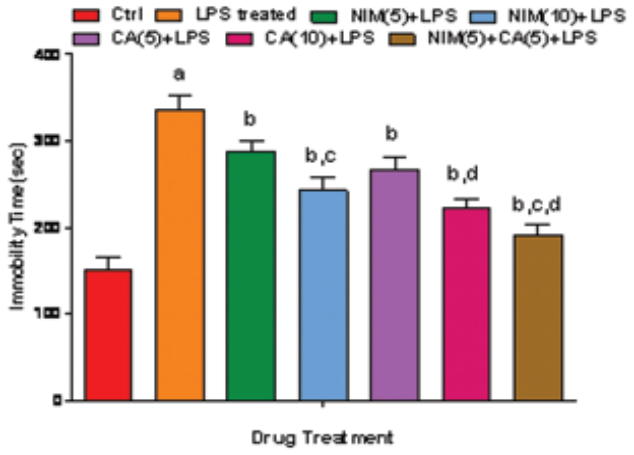

Fig. 1. Effect of nimesulide, caffeic acid and their combination on immobility time in LPS-treated mice on 21st day.
[Immobility time is expressed in seconds as mean ± SEM (n = 6 in each group). [a]p<0.05 as compared to vehicle, [b]p<0.05 as compared to LPS-treated group, [c]p<0.05 as compared to NIM(5)+LPS group, [d]p<0.05 as compared to CA(5)+LPS group (One-way ANOVA followed by Tukey's test)].

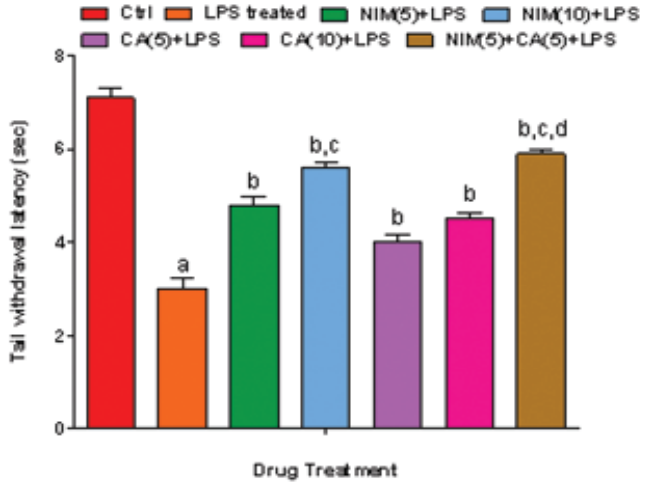

Fig. 2. Effect of nimesulide, caffeic acid and their combination on tail withdrawal latency in LPS-treated mice on 21st day.
[Tail withdrawal latency is expressed in seconds as mean ± SEM (n = 6 in each group). [a]p<0.05 as compared to vehicle, [b]p<0.05 as compared to LPS-treated group, [c]p<0.05 as compared to NIM(5)+LPS group, [d]p<0.05 as compared to CA(5)+LPS group (One-way ANOVA followed by Tukey's test)].

10 mg/kg p.o.) for 21 days significantly (p<0.05) reversed the effect on stress-induced hyperalgesia. Additionally, a lower dose combination of nimesulide (5 mg/kg, p.o.) and caffeic acid (5 mg/kg p.o.) significantly (p<0.05) potentiated their protective effect on stress-induced hyperalgesia in LPS-treated animals, which was significant (p<0.05) as compared to their effect by itself alone. [F(6,41)=48.29, (p<0.05)] (Fig. 2).

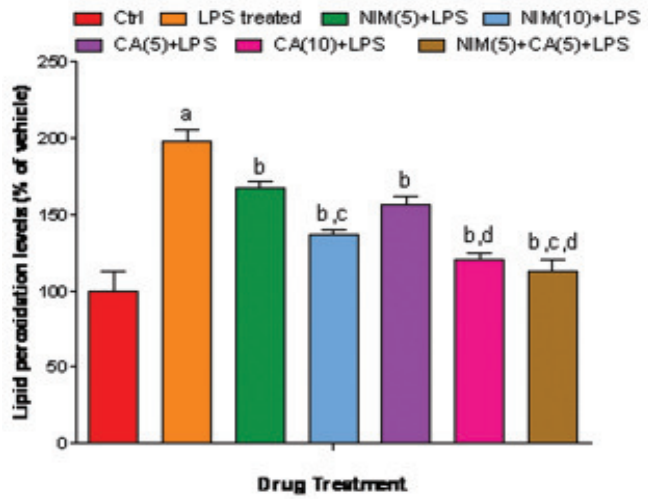

Fig. 3. Effect of nimesulide, caffeic acid and their combination on lipid peroxidation in LPS-treated mice on 21st day.
[MDA levels are expressed as % of vehicle. [a]p<0.05 as compared to vehicle, [b]p<0.05 as compared to LPS-treated group, [c]p<0.05 as compared to NIM (5) + LPS group, [d]p<0.05 as compared to CA(5)+LPS group (One-way ANOVA followed by Tukey's test)].

Effect of nimesulide, caffeic acid and their combination on oxidative stress parameters

LPS challenge caused a marked increase in lipid peroxidation (MDA) levels (Fig. 3) and depleted antioxidant enzymes (GSH and SOD) in the brain of mice (Fig. 4 and 5). Administration of different doses of nimesulide (5 and 10 mg/kg p.o.) or caffeic acid (5 and 10 mg/kg p.o.) significantly reduced MDA levels (Fig. 3) as well as restored the antioxidant enzymes as compared to LPS treated group, (Fig. 4 and 5). Furthermore, when nimesulide (5 mg/kg, p.o.) was combined with caffeic acid (5 mg/kg, p.o.), significantly (p<0.05) decreased the MDA levels [F(6,41)=33.29, (p<0.05)] (Fig. 3), and upregulated GSH levels [F(6,41)=23.59, (p<0.05)] and SOD levels [F(6,41)=33.56, (p<0.05)] as compared to their effect by itself alone (Fig. 4 and 5).

Effect of nimesulide, caffeic acid and their combination on brain nitrite levels

A significant increase in brain nitrite level was seen in LPS challenged group (p<0.05) (Fig. 6). Chronic administration of nimesulide (5 and 10 mg/kg, p.o.) or caffeic acid (5 and 10 mg/kg, p.o.) significantly (p<0.05) reduced the brain nitrite levels in animals challenged with LPS. Furthermore, a lower dose combination of nimesulide (5 mg/kg, p.o.) and caffeic acid (5 mg/kg, p.o.) significantly (p<0.05) reduced the brain nitrite levels as compared to their effect by itself alone [F(6,41)=44.82, (p<0.05)] (Fig. 6).

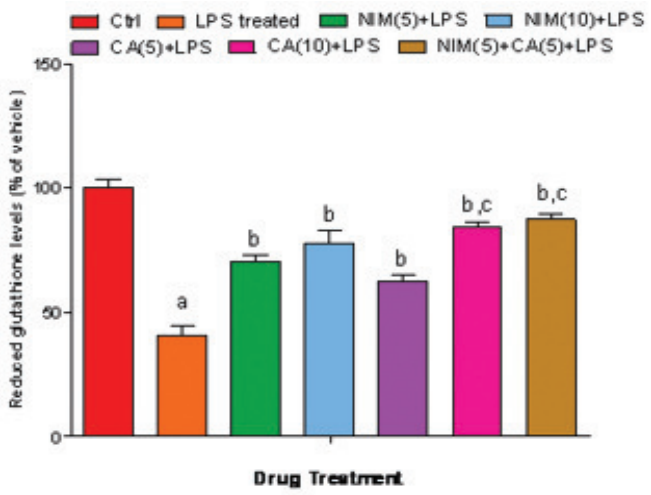

Fig. 4. Effect of nimesulide, caffeic acid and their combination on reduced glutathione levels in LPS-treated on 21st day.
[GSH levels are expressed as % of vehicle. [a]p<0.05 as compared to vehicle, [b]p<0.05 as compared to LPS-treated group, [c]p<0.05 as compared to CA (5) + LPS group (One-way ANOVA followed by Tukey's test)].

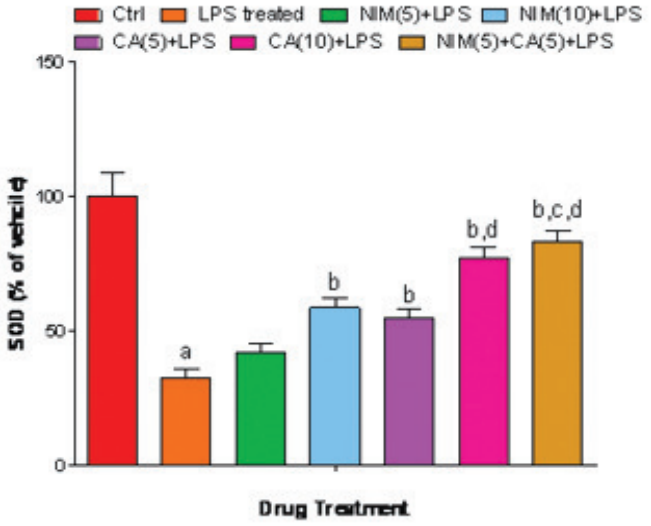

Fig. 5. Effect of nimesulide, caffeic acid and their combination on SOD levels in LPS-treated mice on 21st day.
[SOD levels are expressed as % of vehicle. [a]p<0.05 as compared to vehicle, [b]p<0.05 as compared to LPS-treated group, [c]p<0.05 as compared to NIM (5) + LPS group, [d]p<0.05 as compared to CA (5) + LPS group (One-way ANOVA followed by Tukey's test)].

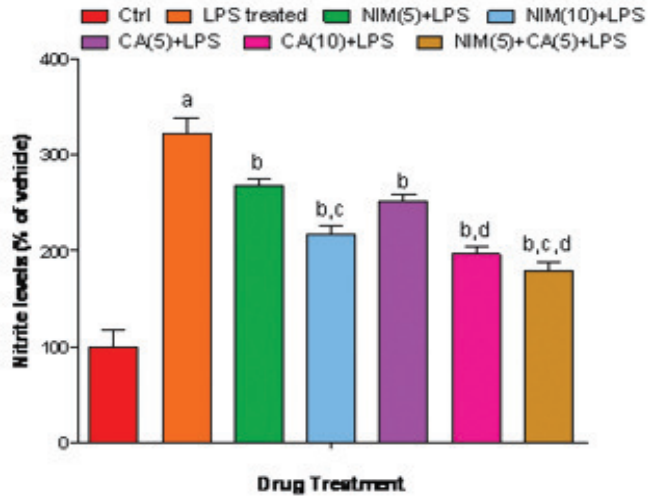

Fig. 6. Effect of nimesulide, caffeic acid and their combination on brain nitrite levels in LPS-treated mice on 21st day.
[Nitrite levels are expressed as % of vehicle. [a]p<0.05 as compared to vehicle, [b]p<0.05 as compared to LPS-treated group, [c]p<0.05 as compared to NIM (5) + LPS group, [d]p<0.05 as compared to CA (5) + LPS group (One-way ANOVA followed by Tukey's test)].

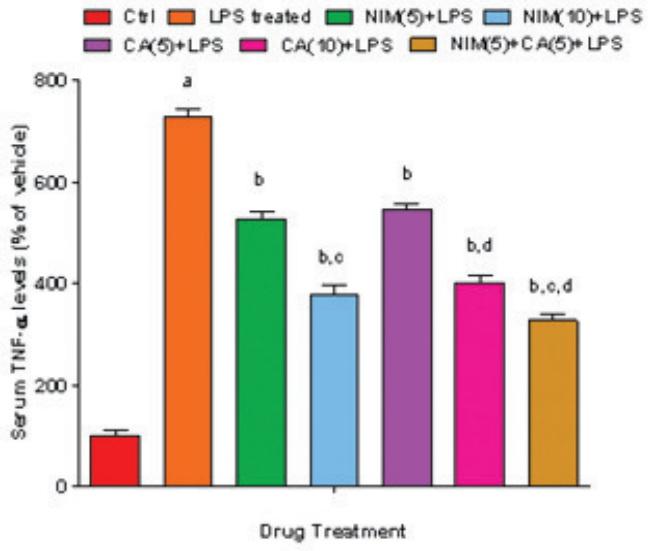

Fig. 7. Effect of nimesulide, caffeic acid and their combination on serum TNF-∝ levels in LPS-treated mice on 21st day.
[TNF-∝ levels are expressed as % of vehicle. [a]p<0.05 as compared to vehicle, [b]p<0.05 as compared to LPS-treated group, [c]p<0.05 as compared to NIM (5) + LPS group, [d]p<0.05 as compared to CA (5) + LPS group (One-way ANOVA followed by Tukey's test)].

Effect of nimesulide, caffeic acid and their combination on serum TNF-∝ level

LPS challenge significantly increased the serum TNF-∝ levels as compared to the vehicle group (p<0.05) (Fig. 7). Chronic administration of different doses of nimesulide (5 and 10 mg/kg, p.o.) or caffeic acid (5 and 10 mg/kg, p.o.) significantly (p<0.05) reduced the serum TNF-∝ levels in LPS treated animals (p<0.05) (Fig. 7). Lower dose combination of nimesulide (5 mg/kg, p.o.)

and caffeic acid (5 mg/kg, p.o.) significantly ($p<0.05$) potentiated their effect by itself [$F(6,41)=61.19$, ($p<0.05$)] (Fig. 7).

Chronic fatigue syndrome is multifactorial and heterogeneous. Previous research emphasizes the strong involvement of immune dysregulation, inflammation, and oxidative stress in the pathogenesis of chronic fatigue syndrome (Morris and Maes 2014). Elevated levels of methemoglobin (MetHb), oxidative damage to DNA, lipids in vastus lateralis muscle samples of CFS patients, and an increase in activity of the antioxidant enzyme system which is a compensatory measure in response to oxidative stress point toward involvement of oxidative stress in the pathogenesis of CFS (Fulle *et al.* 2000, Logan and Wongm 2001). However, it is suggested that elevated peroxynitrite causes mitochondrial dysfunction and lipid peroxidation in CFS. Studies have suggested that increased cytokine level induces the formation of NO that combines with superoxide to form a potent oxidant viz. "peroxynitrite" (Yang *et al.* 2021). Following the above findings, it is probable that targeting oxidative stress and further upregulation of cellular cascade could be beneficial in the management of CFS.

In the present study, mice were challenged with LPS to mimic bacterial infection and induce chronic fatigue-like symptoms and the severity of fatigue was assessed by chronic water-immersion stress (10 min/day) and stress-induced hyperalgesia along with some biochemical parameters for measurement of oxidative stress. LPS acts by binding with TLR4 (Toll-Like receptor 4) and thus promotes the secretion of proinflammatory cytokines in many cell types, especially in macrophages (Raetz and Whitfield 2002, Castaneda *et al.* 2017). Mice were immunologically challenged with LPS and were subjected to chronic water immersion stress of 10 minutes for 21 days. Various biochemical parameters were examined to assess cytokine production, oxidative stress, and nitrosative stress in immunologically activated mice. Since LPS-induced immobility time coupled with hyperalgesia and oxidative stress reached peak levels on day 21. Hence, this study focused on the ameliorative effect of nimesulide, caffeic acid, and their combination for the 21st day. Our model elucidates the long-term behavioral and pathophysiological consequences of immune activation.

LPS challenge significantly induced chronic fatigue-like symptoms in mice as seen by increased immobility time and decreased tail flick latency as seen on the 21st day. Chronic treatment with nimesulide (5 and 10 mg/kg) or caffeic acid (5 and 10 mg/kg) significantly attenuated the LPS-induced behavioral changes in mice. When the combination of these drugs at their respective lower dose (5 mg/kg) was administered, more significant and synergic effects were observed as compared to their effect by themselves. It may be possible that these drugs attenuated the LPS-induced activation of TLR4 and further inhibited the cascade of cellular events. It could be a possible reason for their modulatory effect on LPS-induced behavioral changes in mice.

Caffeic acid has also been shown to inhibit the reduction of SOD and an increase in MDA content in the brain after cryoinjury-induced oxidative stress (Adjimani and Asare 2015). CAPE (caffeic acid phenethyl ester), a derivative of caffeic acid has shown an ameliorative effect in LPS-induced inflammatory stress in rat hippocampal slice cultures and attenuated ischemic reperfusion-induced cerebral lipid peroxidation (Montpied *et al*. 2003, Balaha *et al*. 2021). Nimesulide is a preferential COX-2 inhibitor and is the most prescribed NSAID worldwide (Fanelli *et al.* 2017). Nimesulide has demonstrated a protective effect against oxidative stress and NO production in the hippocampus region of the transient ischemia rat model (Zeynep *et al.* 2017). In another study, nimesulide treatment significantly improved kainite-induced oxidative stress in the hippocampus as revealed by the low level of lipid peroxidation and preservation of endogenous antioxidant capacity (Bishnoi *et al.* 2007). Nimesulide has also been shown to decrease oxidative stress associated with intrastriatal MPTP-induced oxidative stress in rat brains (Gupta *et al.* 2010).

In the present study increased levels of MDA which is the biomarker of lipid peroxidation in the brain of fatigued mice. Further, the levels of GSH and SOD were significantly decreased in immunologically challenged mice. Daily treatment of nimesulide (5 and 10 mg/kg) or caffeic acid (5 and 10 mg/kg) for 21 days significantly ($p<0.05$) attenuated the extent of lipid peroxidation. Brain antioxidant enzymes such as GSH and SOD levels were also significantly restored by nimesulide and caffeic acid as well as their combination at a lower dose. The combination of these drugs at their respective lower doses appears to have potentiated their protective effects in reversing the oxidative stress parameters in LPS-challenged mice.

LPS causes activation of the peripheral immune system and results in the production of various cytokines. If this activation remains persistent, the resultant signaling to the brain increases the severity of the disease (Castaneda *et al.* 2017). It is also reported that endotoxins are the potent stimulators of iNOS and this increased the levels of peroxynitrite, a pro-oxidant (Pall 2013). Elevated levels of peroxynitrite levels are sustained by several potential feedback mechanisms. In this way, a self-perpetuating

vicious cycle is generated leading to a chronic pathological condition. TNF-∝ is also known to regulate peroxynitrite production via PKC activation (Valacchi *et al.* 2018). Thus, it can be proposed that any pharmacological agent that inhibits the expression of iNOS or attenuates the increased peroxynitrite load may prove beneficial in treating CFS. In the present study, LPS-treated mice showed a marked increase in brain nitrite levels and serum TNF-∝ levels. Both nimesulide and caffeic acid reversed these markers in a significant manner. A combination of these drugs also showed more promising results in attenuating the increased nitrite and TNF-∝ level.

Further, the upregulation of key inflammatory mediators (NF-kB, COX-2, iNOS) concludes that CFS is a manifestation of inflammatory pathways along with oxidative and nitrosative stress pathways (Morris and Maes 2014). Increased levels of C-reactive proteins and alpha-2 globulin further confirm the role of inflammation and immune activation in CFS (Nacul *et al.* 2019). Increased expression of COX-2 has already been reported in CFS patients (Gow *et al.* 2009). The above observations suggest the important role of inhibiting these inflammatory biomarkers as well as the potential role of anti-inflammatory drugs in the therapeutic management of CFS clinically.

Some studies suggest that nimesulide acts by preferentially inhibiting the COX-2 enzymatic activity and has also demonstrated potent antioxidant properties (Zeynep *et al.* 2017). Caffeic acid is known for its antioxidant properties and is a potent 5-LOX inhibitor (Boudreau *et al.* 2012, Silva and Lopes 2020). Therefore, it is believed that these drugs under their COX or LOX inhibiting properties in addition to their antioxidant effects produced protective effects against LPS-induced chronic fatigue syndrome in experimental animals. Further research is envisaged to find out the possible mechanism by which these drugs act in alleviating the effects of CFS.

CONCLUSION

The purpose of this research was to correlate inflammation and oxidative stress induced by LPS as an immunogen in the pathophysiology of CFS. Based on the results analysis it is notified that the important role of immune activation and inflammatory and oxidative stress pathways in the pathophysiology of CFS is confirmed by biomarkers. On the other hand, treatment and management of CFS with a combination of nimesulide and caffeic acid at a low dose significantly reduce the symptoms and inflammatory mediators in CFS. This combination acted as an antioxidant and anti-inflammatory via cytokines mediators and also reduced inflammatory biomarkers that were involved in CSF. Further, at low doses this combination could be a valuable therapeutic option for CFS without side effects.

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