Research Article

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NEUROPROTECTIVE EFFECT OF *FENCHONE* ON CHRONIC UNPREDICTABLE MILD STRESS (CUMS) INDUCED NEUROINFLAMMATION

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Abstract

Background: Neuroinflammation has been suspected in the pathogenesis or the progression of the variety of acute and chronic neurological and neurodegenerative disorders including dementia. Aim: The current work is to study the action of fenchone on CUMS induced behavioral changes, oxidative stress and neuronal damage in rats. Methods: Adult male Wistar albino rats were grouped into five six in each. Group I treated with normal saline (0.9% NaCl i.p.), group II treated with normal saline + CUMS, group III treated with Aspirin (200 mg/kg) + CUMS, Group IV treated with fenchone (200 mg/kg) + CUMS (100 µg/kg) and Group V treated with fenchone (400 mg/kg) + CUMS for 27 days followed by single challenged of CUMS to all the groups except control rats. On 28th day onwards, various behavioral assessment such as locomotor activity, cognitive and memory assessment were carried out. Rats were sacrificed, and their brains were extracted and estimated antioxidant levels (SOD, CAT, GSH and TBARS). Results: CUMS treated rats significantly ($P<0.001$) decreased the body weight, locomotor activity, latency period in passive avoidance test and anti-oxidant levels in GSH, SOD and CAT and increased the rectal temperature and lipid peroxidase level compare to control rats pretreated with Aspirin 200 mg/kg rats and fenchone (200 and 400 mg/kg) rats significantly attenuated the CUMS induced behavioral changes, oxidative damage and neuronal damage. Conclusion: Fenchone showed neuroprotective activity due to the presence of Antioxidants.

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INTRODUCTION

CUMS also well known as bacterial endotoxin are biologically active substances, a major cell constituent present on the outer membrane of the cell wall of most Gram-negative bacteria [2,3]. Bacterial CUMS consists of a polysaccharide region that is anchored on the outer bacterial membrane by a specific carbohydrate lipid moiety termed as Lipid A. This lipid A is responsible for the potent immunostimulatory property of CUMS [4,5]. CUMS projects its potency and act as an endotoxin by its greater affinity towards Toll-like receptor 4 (TLR 4) [6]. TLRs initiate key inflammatory responses and also shape adaptive immunity. LPS binds to TLR 4 which interacts with three different extracellular proteins namely CUMS - binding protein (LPB), CD14 (Pattern recognition receptor-cluster of differentiation 14), Myeloid differentiation protein 2 (MD-2), to induce a signaling cascade, leading to activation of NF-κB-dependent induction of pro-

inflammatory cytokines such as TNF-α, IL-1β, IL-6, COX, Prostaglandins and free radicals [4,7-9]. Thus, it is showed that by promoting the formation of free radicals, cytokines and other pro-inflammatory mediators in brain, blood as well as in tissue. CUMS acts as a strong stimulator of potent immune system and produces a potent immunostimulant effect. As an outcome of potent stimulation of immune system by LPS, there results neuroinflammation. Presently the term "Neuroinflammation" is used to describe the inflammatory response originated in the CNS due to the accumulation of glial cells by activating the immune component. But currently there are drugs available for the treatment of neuroinflammation which includes classes of drugs like NSAIDS, Opioid antagonists, selective COX inhibitor, NMDA receptor antagonists and rarely antibiotics [10,11]. But these allopathic medicines on usage, although effective at relieving pain and inflammation, produce undesirable serious side effects. For example, NSAIDS causes gastric irritation, abdominal or stomach pain, nausea, cramping, ringing in ears, confusion [12]. On the other hand, Opioid antagonists also results in unwanted effects like loss of appetite, dizziness, nervousness [13]. As an alternative method of treatment, in order to avoid all major side effects, we have focused our research work on Traditional drugs. These herbal medicines can be used to treat patients with lesser or no side effects as with allopathy.

MATERIALS AND METHODS

ANIMALS

Adult male Wistar rat (180-200 g: 28 rats) were received from the veterinary college, Bangalore, acclimatized for two weeks before experimentation and divided into five groups of six animals each. The rats were housed in colony cages at an aberrant temperature of 25°C ± 2°C with a 12 h light/ dark cycle. The animals had free access to standard pellet diet and drinking water. Behavioral studies were carried out in a quiet room between 9.00 am and 11.00 am to avoid circadian variation. The experimental procedures on animals were in consent with the Committee for the Purpose of Control and Supervision of Experiments on Animals, CPCSEA (Approval Number 878/PO/Re/S/05/CPCSEA/003/2017).

DRUGS AND CHEMICALS

Fenchone (Sigma Aldrich, Mumbai**)** CUMS (Sigma Aldrich, Mumbai), Aspirin (galaxy pharmaceuticals, Tirupati), Thiobarbituric acid (S.D. Fine Chemicals Ltd., Mumbai), and SOD (Adichunchanagiri university, Karnataka) were obtained. All other reagents and chemicals were of analytical grade.

EXPERIMENTAL DESIGN

The animals are divided into five groups with six rats each. The first group (control) received normal saline (0.9% NaCl, p.o.) once daily for 28 days. The second group treated saline for 28 days followed by CUMS challenge. The third group received Aspirin (200 mg/kg p.o) for 28 days followed by CUMS. The fourth group received fenchone (200 mg/kg p.o) for 28 days followed by CUMS. The fifth group received fenchone (400 mg/kg p.o) for 28 days followed by CUMS. All drugs were prepared freshly and given once daily (OD) in the morning and followed the same regimen.

BEHAVIORAL PARAMETERS

SPONTANEOUS LOCOMOTOR ACTIVITY

The spontaneous locomotor activity of Rats was assessed using the Actophotometer, which contains a square arena (30×30 cm) with walls that are fitted with photocells just above the floor level. The photocells were checked before the beginning of the experiment. The drug/vehicle treated rats, were then individually placed in the arena. After a 2 min acclimatization period, the digital locomotor scores were recorded for the next 8 min in a dimly lit room. Test and standard drugs are given in respective doses 30 minutes prior to testing. The activity was performed on day 27th on animals following CUMS and the respective values were expressed [36].

STEP THROUGH PASSIVE AVOIDANCE TEST

The step through passive avoidance apparatus consisted of an illuminated chamber (11.5 cm Â 9.5 cm Â 11 cm) attached to a darkened chamber (23.5 cm Â 9.5 cm Â 11 cm) containing a metal floor that could deliver foot shocks. The two compartments were separated by a guillotine door. The illuminated chamber was lit with a 25 W lamp. Briefly, rat was placed in the dimly lit room containing the apparatus 0.5 h before training to acclimatize to the new environment. Each rat was then placed

individually into the illuminated chamber, facing away from the door to the dark chamber, and allowed to acclimatize for 1 min. As soon as the rat entered the dark chamber, the door was slid back into place, triggering a mild foot shock (0.3 Ma, 50 Hz, 5 s). The rat was then immediately removed from the chamber and returned to its home cage. The latency (time used to change compartment) was recorded. The retention test was conducted 24 h later (day 28 with the rat again being placed in the illuminated chamber and subjected to the same protocol in the absence of foot shock. The upper time limit was set at 300 secs [21]. Dissection and homogenization After the treatment period, animals were sacrificed on 16th day by decapitation under mild anaesthesia. The brains were immediately removed, forebrain was dissected out, and cerebellum was discarded. Brains were put on ice and rinsed in ice-cold isotonic saline to remove blood. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 g for 15 minutes and aliquots of supernatant obtained were used for biochemical estimation.

ESTIMATION OF SUPEROXIDE DISMUTASE (SOD)

The supernatant (500 µL) was added to 0.8 ml of carbonate buffer (100 mM, pH 10.2) and 100 µL of epinephrine (3 mM). The change in absorbance of each sample was then recorded at 480 nm in spectrophotometer for 2 min at an interval of 15 s. Parallel blank and standard were run for determination of SOD activity. One unit of SOD is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto-oxidation. The reaction mixtures are diluted 1/10 just before taking the readings in the spectrophotometer [23].

ESTIMATION OF CATALASE (CAT) LEVEL

The catalase activity was assessed by the method of Aebi [25]. The assay mixture consists of 0.05 mL of supernatant of tissue homogenate (10%) and 1.95 mL of 50 mM phosphate buffer (pH 7.0) in 3 mL cuvette. 1 mL of 30 mM hydrogen peroxide (H2 O2) was added and changes in absorbance were followed for 30 s at 240 nm at 15 s intervals. The catalase activity was calculated using the millimolar extinction coefficient of H2 O2 (90.071 mm mol cm-1) and the activity was expressed as micromoles of H2 O2 oxidized per minute per milligram protein.

ESTIMATION OF GLUTATHIONE REDUCTASE (GSH)

The reaction mixture containing 1 mL of phosphate buffer, 0.5 mL of ethylenediamine tetraacetic acid (EDTA), 0.5 mL of oxidized glutathione 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 min at 30 s intervals. One unit of the enzyme activity was expressed as moles of NADPH oxidized/min/mg protein [22].

ASSAY OF THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS)-MALONALDEHYDE LEVEL

The amount of malonaldehyde was used as an indirect measure of lipid peroxidation and was determined by reaction with thiobarbituric acid (TBA) [23]. Briefly, 1 mL of aliquots of supernatant was placed in test tubes and added to 3 ml of TBA reagent: TBA 0.38% (w/w), 0.25 M hydrochloric acid (HCl), and trichloroacetic acid (TCA 15%). The solution was shaken and placed for 15 min, followed by cooling in an ice bath. After cooling, solution was centrifuged to 3500 g for 10 min. The upper layer was collected and assessed with a spectrophotometer at 532 nm. Results were expressed as nanomoles per mg of protein. The concentration of MDA was calculated per mg of protein.

STATISTICAL ANALYSIS

The results were expressed as mean ± SEM. Statistical differences between the mean of various groups were analysed by using variance followed by the Turkey multiple comparison test and also Dunnet's test. Differences were considered significantly at $P<0.05$.

RESULTS

EFFECT OF FENCHONE ON CUMS INDUCED ALTERATIONS IN LOCOMOTOR ACTIVITY

CUMS subjected rats significantly ($p<0.001$) decreased the locomotor activity in the actophotometer when compared to control rats.

Pretreatment with Aspirin 200 mg/kg along with CUMS significantly (p<0.001) increased the locomotor activity in the actophotometer when compared to CUMS subjected rats. Pretreatment with fenchone (200 mg/kg) and fenchone (400 mg/kg) significantly(p<0.001) increased the locomotor activity in the actophotometer when compared to CUMS subjected rats.

Effect of fenchone on CUMS induced memory impairment in passive avoidance test. The effect of fenchone on long-term memory was investigated in the step-through passive avoidance test. During the training session (Day 1), there was no significant difference between any groups. However, there was a significant difference in the retention latency between the groups. CUMS subjected rats showed a significantly (P<0.001) by administration of fenchone 200 and 400 mg/kg. Aspirin treated group significantly increased the latency period during retention test when compared to fenchone treated group. There was no significant difference in the retention latency period between the doses of fenchone 200 mg/kg and fenchone400 mg/kg.

EFFECT OF FENCHONE ON ANTI-OXIDANT LEVEL

EFFECT OF FENCHONE ON CUMS INDUCED ALTERATIONS IN SOD LEVEL

CUMS treated rats produced significantly (P<0.001) decrease in the level of SOD compared with control group. Aspirin treated group showed significantly (P<0.001) increased the level of SOD when compared with CUMS subjected group. Fenchone (200 and 400 mg/kg) restored significantly (P<0.001) the level of SOD but the better antioxidant effect was obtained from fenchone of 400 mg/kg than the fenchone (200 mg/kg).

EFFECT OF FENCHONE ON CUMS INDUCED ALTERATIONS IN CAT LEVEL

CUMS subjected group significantly (P<0.001) decreased the level of CAT when compared to control group. Aspirin treated group significantly (P<0.001) increased the CAT level when compared to CUMS subjected group but less increase when compared to control and Aspirin treated group. There was no significant difference on the activity between the fenchone (200 mg/kg) and fenchone (400 mg/kg) treated groups.

EFFECT OF FENCHONE ON CUMS INDUCED ALTERATIONS IN GSH LEVEL

CUMS subjected rats significantly (P<0.001) decreased the level of GSH when compared to control rats. Aspirin treated rats significantly (P<0.001) increased the GSH level when compared with the CUMS subjected rats. Fenchone (200 and 400 mg/kg) treated rats increased the level of GSH produce significantly(P<0.001) when compared with CUMS subjected rats. There was no significant difference in the level of GSH between fenchone 200 mg/kg and 400 mg/kg.

EFFECT OF FENCHONE ON CUMS INDUCED ALTERATIONS IN TBARS LEVEL

CUMS subjected group significantly (P<0.001) increased the level of TBARS when compared with the control group. Aspirin treated group decreased the TBARS level significantly (P<0.001) when compared with CUMS subjected rats. Fenchone (200 and 400 mg/kg) administered group significantly decreased the level of TBARS when compared to CUMS treated group but the effect is less significant than Aspirin treated group. Fenchone 400 mg/kg administered group exerted its better anti-oxidant activity by decreased the level of TBARS when compared to fenchone (200 mg/kg).

Table 01: Effect of fenchone on Locomotor Activity

S.NO	GROUPS	LOCOMOTOR SCORES
1	Control	409.5± 3.722
2	CUMS	259.7±3.989
3	Aspirin (200mg/kg p.o)	379±6.129
4	Fenchone(200mg/kg p.o)	327± 40274
5	Fenchone(400mg/kg p.o)	303.3±4.745

The values expressed are one way ANOVA followed by Dunnett's test.

Table 02: Effect of fenchone on CUMS induced alterations in SOD, CAT, GSH and TBARS levels.

TREATMENT	SOD	CAT	GSH	TBARS
Control	7.15±0.48	4.02 ± 1.33	42.22 ± 6.94	2.05 ± 0.28
CUMS	2.82±1.23 $	1.59 ± 1.02 $	25.39 ± 2.97 $	4.89 ± 0.86 $
Aspirin (200mg/kg p.o)	6.94±2.05*	3.52 ± 1.58 *	36.14 ± 8.76 *	2.01 ± 0.26 *
Fenchone(200mg/kg p.o)	5.15±0.86*	2.38 ± 1.36 #	29.94 ± 6.54 @	2.43 ± 0.57 #
Fenchone(400mg/kg p.o)	6.39±0.96*	2.48 ± 1.05 *	28.87 ± 7.54 #	2.02 ± 0.11 *

Values are expressed mean ± SEM. $P<0.001 compared to control rats
*P<0.001, #P<0.01 and @P<0.05 compared CUMS subjected rats.

Table 03: Effect of fenchone on CUMS induced memory impairment in passive avoidance test

TREATMENT	ACQUISITION TRAIL	RETENSION LATENCY
Control	15.50 ± 1.08	78.67 ± 3.87
CUMS	26.67 ± 0.66	24.17 ± 1.16 a
Aspirin (200mg/kg p.o)	16.33 ± 0.61	111.7 ± 4.82 *
Fenchone(200mg/kg p.o)	19.67 ± 1.20	97.33 ± 2.24 *
Fenchone(400mg/kg p.o)	18.33 ± 1.49	92.00 ± 1.69 *

Values are expressed mean ± SEM. aP<0.001 compared to control rats.
*P<0.001 compared to CUMS subjected rats.

DISCUSSION

The central findings of the current study reveal that fenchone attenuates CUMS induced sickness behavior on rats. CUMS can bind with TLR4 leading to the activation of NF-κb-dependent induction of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and COX which leads to sickness behavior. These findings replicate the results of many previous studies, which demonstrated that activation of the immune system by CUMS induces a Sickness behavior which refers to a coordinated set of behavioral changes that develop during the course of raised systemic inflammation [26] and include reduction in appetite and body weight, suppression of locomotor, exploratory, and social activity, fatigue and malaise, impairment in cognitive abilities, reduced libido and sexual behavior, anhedonia and impairment in motor behavior [28-30]. In our present study CUMS subjected rats showed decrease in body weight when compared to control rats due to stress. The body weight was increased to rats were treated with Aspirin and fenchone (200 and 400 mg/kg) results from anti-stress activity with active food intake when compared to CUMS treated group.

CONCLUSION

play their protective roles via increased SOD and catalase levels, restoration of GSH, decreased MDA levels and also protects of neurons against ROS as antioxidant activities. play their protective roles via increased SOD and catalase levels, restoration of GSH, decreased MDA levels and also protects of neurons against ROS as antioxidant activities. The fenchone may elicit neuroprotective activity may be due to the presence of antioxidant profile. Fenchone play a protective role via increased SOD and catalase levels, restoration of GSH, decreased MDA levels and also protects of neurons against ROS as antioxidant activities.

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