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Research Article

EFFECT OF LYCOPENE ON CHRONIC MILD STRESS-INDUCED HYPERLIPIDEMIA IN WISTAR ALBINO RATS

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ABSTRACT

Objective: Chronic mild stress is the most valid model in inducing depression in rodents. In this method, rats were subjected to CMS for 6 weeks of stress.

Methods: In this method, rodents were subjected to a series of mild stressors for CMS for six weeks in an unpredictable manner.

Results: Biochemical and pathological changes were observed. Lycopene treatment at 10 mg/kg and 20 mg/kg could revert these biochemical changes. Histopathological studies showed there is a neuronal loss in CMS and CMS+Vehicle groups. Lycopene treatment reverted this condition.

Conclusion: Lycopene treatment might revert this biochemical change by inhibiting a rate-limiting enzyme, HMG-CoA reductase. Histopathology of the brain revealed that rats subjected to chronic mild stress showed a decreased neuronal loss in the hippocampus. Lycopene treatment showed a neuroprotective effect against CMS-induced neuronal loss.

Keywords: Chronic mild stress, Lycopene, Hyperlipidemia.

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INTRODUCTION

Depression is the most common mood disorder affecting a large part of the world population. The incidence of depression is seen in every one in five persons and presents with symptoms of depression, such as sleep deprivation, anhedonia, suicidal intention. According to the world health organization (WHO), depression will be the second leading cause of disease in human beings by 2020 [1]. The prevalence of depression ranges from 20% to 25% in women and 7-12% in men. The overall prevalence of depression in the geriatric population varies between 10%-20% [2]. According to WHO it is estimated that 15% of adults aged above 60 suffering from mental disorders like depression and dementia are at more risk of death. Lycopene is a carotenoid found extensively in fruits. Lycopene is a potent antioxidant and a free radical scavenger in presence of β-ionic ring in its structure, and possessing is the highest antioxidant among carotenoids [3]. Lipophilic nature of lycopene make it free assessable to cross the blood brain barrier and showed protective against various oxidative diseases generated by reactive oxygen species cause a modification in DNA functions [4]. Inactivation of oxidative enzymes contributes to degenerative neuronal diseases like Alzheimer's disease, parkinsonism.

The CMS model of depression was developed by Willner *et al*. and is the most reliable model in inducing depression-like behavior in rodents and had multiple effects on neurobiological and biochemical activities [5]. According to the vascular hypothesis of depression, hypercholesterolemia can be a risk factor for inducing depression [6]. Similarly, long-term administration of multiple mild stressor exposure can dispose to a depressive state. In our present study, we aimed to assess biochemical changes in lipid profile, typically present during induction of CMS. To our knowledge lycopene effect on CMS-induced biochemical changes is not studied extensively. Glucocorticoids released during stress have a deleterious impact on the brain, particularly the hippocampus, rich in corticosteroid receptors and sensitive to adrenal steroids. The hippocampus region undergoes morphological changes like atrophy, loss of neurons and further reduced hippocampal volume in response to stress[7]. Stress can imply the brain's ability to regulate both physiological and biochemical changes to stress [8]. According to vascular depression theory, cholesterol-lowering drugs improve antidepressant drug efficacy by lowering cholesterol levels in depression patients [9]. Hypercholesteremia tends to decrease membrane fluidity and causes modifications, resulting in poor treatment outcomes in different antidepressants [10].

METHODS

Experimental animals

The experiments were conducted as per CPCSEA guidelines. Forty-two adult male Wistar albino rats weighing between (240±10) grams were selected. Animals were kept under standard conditions, and water was supplied ad libitum throughout the study. The experimental protocol was conducted after getting clearance from the institutional animal ethical committee.

Drugs

Standard drug Imipramine hydrochloride was purchased from Sigma–Aldrich, and test drug lycopene powder was obtained as a free sample from parry nutraceuticals private limited Pune India. Lycopene is suspended in corn oil as a vehicle. The control group will receive saline water. Lycopene doses of 5 mg/kg, 10 mg/kg, 20 mg/kg, and standard drug imipramine at a dose of 10 \mg/kg were selected based on previous literature.

Experimental design

Animals were acclimatized into the experimental room a week before starting the experiment. Rats were trained to consume a 1% sucrose solution twice a week in which animals were fasted for 14 h of food and water deprivation, followed by a 1 h sucrose preference test. Based on the sucrose preference test, 42 Wistar albino rats were randomly divided into the control group (n=6) and the CMS-induced group (n=36). Control group rats were placed in a separate room free from contact with other stressor groups. CMS induced group further divided into CMS, CMS+Vehicle, CMS+ Imipramine group and CMS+Lycopene in three graded doses (i.e., 5 mg/kg, 10 mg/kg and 20 mg/kg) (n=6).

All the stressed group's animals are subjected to a serious stressor unpredictably to prevent dependence for the duration of 6-weeks. The study protocol consists of eight stressors described in Table 1.

The control group did not expose to a chronic mild stress regimen; each stressor lasted for 10–14 h. Animals are deprived of food and water 24 h before the test to avoid any nonspecific influence of diet on sucrose consumption. The bodyweight of each animal was noted weekly before the sucrose preference test. At the end of the behavioral studies, blood was collected from all the groups for biochemical estimations. Blood samples were taken following a 10-12 h fast. Blood was allowed to clot at room temperature, and serum was obtained immediately by centrifugation at 3500 revolutions per minute (rpm) for 10 min. Serum was aliquoted into plastic tubes and stored at 27°C until assayed, then euthanized using Co2 asphyxiation and subjected to full detailed gross necropsy. Brain collected from all the groups. Brain hippocampal region were dissected out rapidly for studying further histopathology studies.

RESULTS

CMS group rats showed an altered serum lipid profile as an evidence of increase in total cholesterol [F(6,35)=48.28; p<0.000]; Triglycerides [F(6,35)=13.49; p<0.000]; HDL-cholesterol [F(6,35)=12.74; p<0.000], in comparison with control group. Lycopene supplementation significantly decreased the elevated serum levels of cholesterol, triglycerides and increased HDL levels (Table 2).

At the end of the experimental period, rats from each group were euthanized using CO_2 asphyxiation and subjected to full detailed gross necropsy. The brain was collected from all animals of all dose groups and preserved in 10% neutral buffered formalin. After 48 h of fixation, all fixed tissues were processed and embedded in paraffin wax. Sectioned approximately 3–5 µm (approx.) thick and stained with hematoxylin and eosin for histopathological examination. The following grading system was used for histopathological examination in the study. (1) Minimal, (2) Mild, (3) Moderate, (4) Marked, (5) Severe [11].

DISCUSSION

Long-term stress in humans disposes to a depressive state. Loss of appetite and disturbance in sleeping patterns are characteristic features of depression. Stress is believed to be an important etiological factor in depression. The CMS model is the most valid model in inducing depression in rodents, where they are inflicted with various potential stressors over 6-weeks. According to a literature search for chronic mild stress induces metabolic disturbances by altering serum lipid profiles. Stress can alter the serum lipid profile. There is growing evidence that stress triggers distinct biochemical parameters in humans and animals [12]. In the present study, we aimed to determine changes in the serum lipid levels in rats subjected to CMS. Hypercholesterolemia increases the risk of developing psychiatric disorders like anxiety and depression by destroying the brain's neurotransmitters, and it causes an imbalance in the neurotransmitter levels [13]. Rats subjected to the CMS procedure elicited a significant change in the serum lipid profile in the CMS group compared to the control group [14,15]. Chronic mild stress might induce phosphorylation of the HMG-CoA reductase enzyme, a rate-limiting step in cholesterol synthesis, thereby increasing the serum lipid levels. In our present study, CMS induced group showed a significant increase in serum cholesterol triglycerides, and reduction in high density lipoprotein levels. However, imipramine-treated groups failed to alter elevated serum lipid profiles probably because of weight gain associated with tricyclic antidepressants, and these findings are similar to our bodyweight estimations [16]. Administration of lycopene at a dose of 10 mg/kg and 20 mg/kg significantly reversed hyperlipidemia. According to earlier reports, lycopene inhibits HMG-CoA reductase, a rate-limiting enzyme in cholesterol synthesis [17].

Table 1: CMS stress protocol

S. No.	Types of stressor	Procedure
1	Soiled Cage	In which 250 ml of plain water is poured into the sawdust
2	Cage tilting	Tilt the cages backward (~45°)
3	Grouped housing	In which randomly changing the partners
4	Intermittent illumination	Switch the room lights on and off every~2 h
5	Food and water deprivation	Food and water deprivation
6	Water deprivation	Deprivation of water
7	Crowed grouping	Two rats per cage
8	Food deprivation	Deprivation of food

Histopathology of the brain in the control group revealed normal hippocampal neurons in the CA1 region (Fig 1). However, in CMS and Vehicle+CMS groups showed a mild neuronal loss in the CA1 region of the hippocampus compared with control group animals. Imipramine drug showed no detectable neuronal loss compared with CMS and CMS+Vehicle groups. Lycopene 5 mg/kg and 10mg/kg revealed a minimal neuronal loss in the hippocampus compared

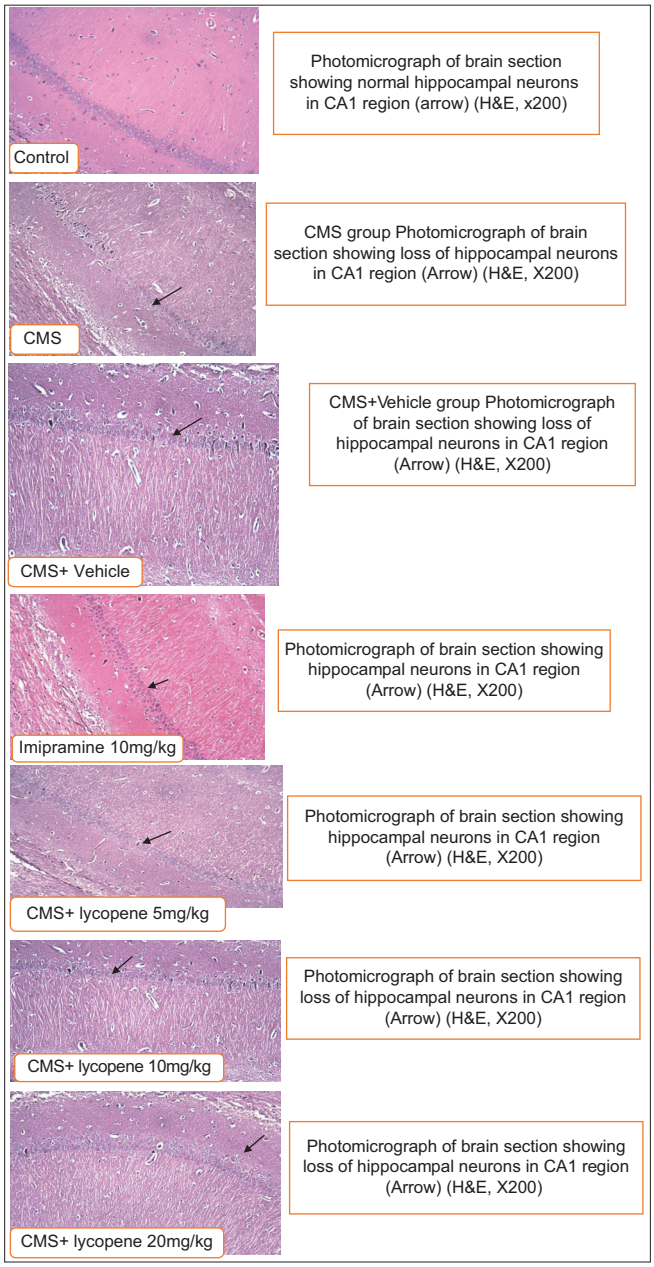

Fig. 1: Histopathology of hippocampal

Table 2: The Effect of lycopene and imipramine treatments on serum total cholesterol levels in control and CMS induced rats

Serum lipid profile			
Groups	**Cholesterol (mg/dL)**	**Triglycerides (mg/dL)**	**HDL-cholesterol (mg/dL)**
Control	136.6±0.9	89.1±1.6	33.0±0.7
CMS (only stress)	163.3±3.1	107.6±2.4	26.5±0.6
CMS+Vehicle	165.8±1.7	104.5±3.5	27.8±1.5
CMS+Imipramine10 mg/kg	161.6±0.8	103.6±2.1	25.5±0.4
CMS+Lycopene 5 mg/kg	157.3±2.8	100.3±1.0	24.1±1.1
CMS+Lycopene 10 mg/kg	142.3±1.4	92.1±0.7	32.5±0.4
CMS+Lycopene 20 mg/kg	132.3±1.6	87.0±1.0	33.5±1.3

with CMS and CMS+Vehicle groups. Lycopene 20 mg/kg showed no detectable neuronal loss in the CA1 region of the hippocampus compared with lycopene 5 mg/kg and lycopene 10 mg/kg. Lycopene treatment might regulate the HPA-axis, thereby decreasing the corticosterone release, which might be preventing the adverse effect on the brain. Lycopene treatment also averts changes in lipid profile, thereby preventing diminished monoamine levels responsible for the regulation of mood.

CONCLUSION

In our study, six weeks of chronic mild stress induced biochemical changes as evident by hyperlipidemia and neuronal loss in the hippocampus compared to control. Lycopene showed neuroprotective effects at a 20 mg/kg dose and did not show a detectable neuronal loss in the hippocampus compared to the CMS group. Further studies are to be conducted for the analysis of neurotoxicity.

AUTHORS' CONTRIBUTIONS

Dr. Deepak Shankarappa was involved in the planning and execution of the study. Venkata Naveen Kumar P was involved in writing, reviewing the manuscript, and executing the study. Dr. Lourdu jafrin A, involved in editing the manuscript, reference management, and overall inputs. Dr. Somasundaram G helped correct typographical and grammatical errors in the manuscript.

CONFLICTS OF INTEREST

The authors affirm no conflicts of interest, finance, or otherwise.

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