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Original Article

Pharmacology Section

Effect of Lycopene on Antioxidant Status and Serum Corticosterone in Wistar Rats Subjected to Chronic Mild Stress

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ABSTRACT

Introduction: Stress is one of the major causative factors in developing depression. Depression is described as a stress-related disorder which often gets precipitated with chronic stress. Lycopene unlike other carotenoids has the highest antioxidant property and is readily soluble in lipids and crosses the blood-brain barrier, is widely found in fruits and vegetables. Epidemiological data shows that lycopene can prevent and treat various diseases like cardiovascular and neurological disorders. Chronic Mild Stress Method (CMS) is the most valid animal model in inducing depression in rodents. Imbalance in the activities of both oxidant and antioxidants are the important etiological factors in depression. This therapeutic effect can be potential too for various disorders.

Aim: To investigate the possible beneficial effects of lycopene on the antioxidant enzyme activity, serum corticosterone levels and adrenal gland weights in Wistar albino rats subjected to chronic mild stress.

Materials and Methods: The study group consisted of 42-male Wistar rats divided into 7 groups with each group consisting of 6 animals. Control group, CMS group, Vehicle group and Imipramine (10 mg/kg) as a standard drug and lycopene with varying doses of (5, 10, 20 mg/kg) as the test drug. Blood samples were used in estimating serum corticosterone and brain tissues were used in estimating brain antioxidant enzyme activity. Differences in the groups were analysed statistically by one way-ANOVA, followed by post-hoc test was used to find out significant differences between the control and CMS group.

Results: The data analysis showed that CMS could show a significant increase in serum corticosterone levels in the CMS group (only stress) in comparison with the control group ($p<0.05$). Significant decrease in the levels of SOD, CAT and GSH levels and an increase in the MDA levels were observed in CMS and vehicle-treated group rats. Lycopene supplementary doses of 10 mg/kg and 20 mg/kg administration for 6-weeks significantly improved antioxidant enzyme activity.

Conclusion: The study results, support that chronic mild stress induces oxidative stress in the rodents. There was a decrease in SOD activity and an increase in the serum corticosterone levels and adrenal gland weight in CMS and vehicle groups in comparison to the control groups. Lycopene supplementation reverses this state and shows antidepressant-like activity.

Keywords: Brain derived neurotrophic factor, Depression, Hypothalamic pituitary axis

INTRODUCTION

Depression is a psychiatric disorder characterised by loss of appetite, irritability, lack of concentration, disturbance in the sleep [1]. Molecular basis for depression remains unclear. Alterations in the cellular mechanism can induce these disorders. Aetiology of depression that includes depletion of nerve growth factors like BDNF, a decrease in monoamines like dopamine, serotonin, noradrenaline disturbances in HPA-axis and atrophy of cells that are observed in depressant patients. Chronic stress induces depression by changing both physiological and biochemical parameters antidepressants reverse this condition. Cell death is regulated by both neuronal activity and tropic support lack of this activity can induce apoptosis [2]. Stress decreases neurogenesis in the hippocampus region and a decline in hippocampal volume and functions of HPA-axis were often detected in the depressed patient [3,4]. Patients treated with the antidepressant drug and electroconvulsive therapy stimulate neurogenesis [5]. Chronic stress induces atrophy and neurogenic changes such as decreased proliferation, down-regulation of granule cell in response to stress. Hippocampal region of the brain is more abundant in the glucocorticoids receptors and sensitive to various kinds of a stressor which reduces the proliferation of new cells stress activates the HPA-axis and also stimulates the sympathetic nervous system which results in a change in the physiological response which can induce depression [6]. Chronic mild stress caused a reduction in hippocampal volume, because of disturbances in negative feedback mechanism in HPA-axis, the subsequent release of corticosteroids results in the development of resistance to glucocorticoid receptors [7]. CMS model showed a consistent change in HPA-axis and increase in body weights and adrenal gland size.

Chronic stress induces atrophy of neurons and neurogenic changes by decreasing proliferation rate and down regulating of granule cell genesis in response to stress [5]. Antidepressant drug and electroconvulsive therapy revert this by stimulating neurogenesis. Increase in oxidative stress plays a major role in the pathogenesis of depression [8]. Several studies demonstrate stress can alter antioxidant status in the brain [9,10]. Increase in plasma reactive oxygen species are associated with elevated antioxidant enzyme activities is a severe form of depression. i.e., the SOD brain contains a high content of peroxidizable fatty acids. Lipid peroxidation displays a decrease in mitochondria functions, causing damage to DNA, and altered cogitative functions in the brain [11]. Impairment in neurogenesis is the deleterious effect of oxidative stress [12]. The brain utilises high content of oxygen and also more susceptible to oxidative stress. Chronic mild stress causes a disturbance in both antioxidants and pro-oxidant enzyme activity in hippocampus and shifted towards the pro-oxidant activity. The brain is more susceptible to oxidative damage due to its high utilisation of oxygen and limited amounts of antioxidant capacity making it venerable to oxidative damage and cellular dysfunction and promote apoptosis [2]. Lycopene is a potent antioxidant and a free radical scavenger in the presence of the β-ionic ring in its structure and possessing highest antioxidant among carotenoids and lipophilic in nature make it free assessable to cross the Blood-Brain Barrier (BBB) and showed

protective against various oxidative diseases due to generation of reactive oxygen species causing modification in DNA functions, inactivation of oxidative enzymes contribute to neuronal degenerative diseases like Alzheimer's disease, parkinsonism. Similarly, long-term administration of multiple mild stressor exposures can dispose of a depressive state.

The present authors aimed to determine changes in serum corticosterone and adrenal gland weight and antioxidant status, typically present during induction of CMS and to test the effects of lycopene, in chronic mild stress induce oxidative stress. To the best of authors knowledge, there was no data available on the effects of lycopene in CMS-induced, oxidative stress and changes that are typically presented during stress.

MATERIALS AND METHODS

Experimental Animals

Animals were brought in to the laboratory room a week before experimentation for acclimatisation. The experiments were conducted as per CPCSEA guidelines. Forty-two adult male Wistar albino rats weighing between 240±10 gm were selected. Animals were kept under standard conditions of a 12 hours light/dark cycle with the standard chow feed and water were supplied *ad libitum* throughout the procedure with controlled temperature (22±2°C) and humidity (40%-60%). All the experiments were carried out between 09.00 and 16.00 hours. The Sample size was calculated based on the previous literature [13]. The experimental protocol was conducted after getting clearance from the Institutional Animal Ethical Committee of Sri Rama Chandra Institute of Higher Education and Research (SRIHER), Chennai, Tamil Nadu, India. During the period of August 2017-December 2017 (IAEC/52/SRU/563/2017).

Drugs and CMS Experimental Design

Imipramine hydrochloride was purchased from Sigma-Aldrich and lycopene DC powder 10% product code LYP-03-C was from parry nutraceuticals limited Pune India. Lycopene is a lipid-soluble carotenoid and it was dissolved in corn oil, and used as a vehicle for administrating lycopene and imipramine once daily orally for 6-weeks. Control and CMS group had received 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 in accordance with previous literature [13,14]. CMS and vehicle group received an equal volume of corn oil and the control group received only saline water [Table/Fig-1].

Experimental group	Dose	Treatment
Control	Saline	No Stress
CMS	Saline	Stress
CMS+Vehicle	Corn oil	Stress+vehicle
CMS+Imipramine	10 mg/kg bw^{-1}	Stress+Treatment
CMS+Lycopene	5 mg/kg bw^{-1}	Stress+Treatment
CMS+Lycopene	10 mg/kg bw^{-1}	Stress+Treatment
CMS+Lycopene	20 mg/kg bw^{-1}	Stress+Treatment

[Table/Fig-1]: Experimental Design.

Fourty-two Wistar albino rats were randomly selected and divided into seven groups of six each.i.e control group, CMS group, vehicle group, imipramine group, and lycopene.

CMS protocol: CMS procedure have carried out as per described by Willner P, with minor modifications [15]. On completion of baseline sucrose preferences tests, animals were divided into two groups control group and to-be-stressed group. Control group animals were placed in a separate room and to-be stressed groups were subjected, to a series of stressor in an unpredictable manner to prevent dependence continuously for six weeks. Control group were free from stress and housed separately. Each stressor lasted for 10-14 hours.

Sucrose preference test (PST): Sucrose consumption test was performed as earlier described by Luo DD et al., [16]. Wistar rats were trained to consume (1%) palatable sucrose solution for an hour by depriving water and food 14 hours before conducting the test. All the animals were allowed to consume water for a period of one hour at the end of one hour task. Sucrose consumption volumes were recorded difference in the bottle weight before and after gives an amount of sucrose consumption.

Collection of blood and brain tissue: Immediately after completion of behavioural tests, rats were made to rest a day. The following day blood was collected, from orbital sinus under anaesthesia from all the groups. Collected blood was centrifuged to separate the serum and plasma. After completion of blood collection, animals were subjected to decapitation. Brain regions were dissected out rapidly and the hippocampus region was separated from the brains and stored for further studies.

Estimation of serum corticosterone and adrenal gland weight: After separation of serum from plasma, the serum was used in estimating corticosterone levels by commercially available ELISA kit (Cloud-Clone, USA) and all the samples were measured at 450 nm on Elisa reader as per user's manual, and values were expressed in pg/mL. Adrenal gland were dissected out through laparotomy, and weigh in the analytical balance. Measurement of adrenal gland size was used in determining the intact of HPA-axis [17].

Antioxidants

Superoxide dismutase: Superoxide dismutase (SOD) activity was estimated in the brain and expressed in U/mg protein as per described by Marklund S and Marklund G [18] and its colour intensity was recorded by spectrophotometry at 560 nm. Briefly, it contains a mixture of tissue homogenate with buffers such as sodium pyrophosphate phenazine methosulphate and nitroblue tetrazolium. The reaction mixture was heated and incubated for 90 seconds at 30°C with glacial acetic acid to stop the reaction.

Lipid peroxidation: Lipid peroxidation was estimated in the brain as per Devasagayam TP and Tarachand U, [19]. In this method, thiobarbituric acid reacted with malondialdehyde and produced a coloured product which was measured at 532 nm using a spectrophotometry. The content of MDA in the samples was expressed in nmol MDA/mg protein.

Catalase activity: Catalase assay was carried out as described by Sinha AK, [20]. Un-reacted H_2O_2 was measured by its ability to reduce dichromate in acetic acid solution and absorbance at 570 nm spectrophotometrically. Catalase activity was expressed in U/mg protein.

Glutathione Peroxidase (GPx): GPx activity was carried by the method of Rotruck JT et al., [21]. The colour developed was read at 412 nm against the blank. The enzyme activity was expressed as U/mg protein.

STATISTICAL ANALYSIS

Statistical analysis was done to find out the difference in statistical significance between the groups. Graph Pad Prism version 5.0 was used in analysis and p-values less than 0.05 and was considered statistically significant. Analysis of variance (ANOVA) was used to compare the groups. Data were presented as Mean±SD.

RESULTS

Effect of Lycopene on Serum Corticosterone

In the present study, the CMS group showed an increase in the serum corticosterone levels in comparison with the control group which was statistically significant {F(6,35)}=13.30, p<0.05. Lycopene supplemented dose of 10 mg/kg and 20 mg/kg showed a significant decrease in the serum corticosterone levels {F=(6,35)}=13.30. p<0.05. In comparison to the CMS group. Lycopene supplemented

dose of 5 mg/kg did not show any significant change in the serum corticosteroids levels {F(6,35)}=13.30 p>0.05 [Table/Fig-2].

Groups	Serum corticosterone
Control	81.6±3.6
CMS (only stress)	103.0±10.0**
CMS+Vehicle	106.1±3.5**
CMS+Imipramine 10 mg/kg	84.0±7.7#
CMS+Lycopene 5 mg/kg	98.3±7.9
CMS+Lycopene 10 mg/kg	88.0±4.0##
CMS+Lycopene 20 mg/kg	86.0±6.4##

[Table/Fig-2]: Effect of lycopene and imipramine treatments on serum corticosterone in control and CMS-treated rats.
The results are expressed as Mean±SD, (n=6) rats in each group. Control vs. CMS and CMS vs. lycopene (5, 10, 20 mg/kg). Statistical significance were determined by one way ANOVA followed by *Tukey's*,*p<0.05, **p<0.01,***p<0.001 versus control and, #p<0.05, ##p<0.01, ###p<0.001 versus CMS

Effect of Lycopene on Adrenal Gland Weight

The CMS group showed hypertrophy in the adrenal gland in comparison to the control group by increasing the adrenal glands weight significant {F(6,35)}= 11.06. p<0.05. Supplemented dose of 20 mg/kg significantly prevent the hypertrophy changes in the adrenal gland. {F(6,35)}=11.06. p<0.05. Lycopene supplementation dose of 5 mg/kg and 10 mg/ kg did not showed any significant changes (in the adrenal gland weight) {F(6,35)}=11.06; p>0.05 [Table/Fig-3].

Groups	Adrenal gland (Weight in grams)
Control	0.023±0.004
CMS (only stress)	0.045±0.01**
CMS+Vehicle	0.046±0.08**
CMS+Imipramine 10 mg/kg	0.025±0.005#
CMS+Lycopene 5 mg/kg	0.038±0.007
CMS+Lycopene 10 mg/kg	0.030±0.006#
CMS+Lycopene 20 mg/kg	0.027±0.005##

[Table/Fig-3]: Effect of lycopene and imipramine treatments on adrenal glands size in control and CMS-treated rats.
The results are expressed as Mean±SD, (n=6) rats in each group. Control vs. CMS and CMS vs. lycopene (5, 10, 20 mg/kg). Statistical significance were determined by one way ANOVA followed by *Tukey's*
*p<0.05, **p<0.01, ***p<0.001 versus control and, #p<0.05, ##p<0.01, ###p<0.001 versus CMS

Effect of Lycopene on Antioxidant Enzymes Activity in Brain

Chronic mild stress exposure induced a significant reduction in the antioxidant enzyme activity like SOD, Glutathione Peroxidase (GPx) and Catalase (CAT) activity and increase in Malondialdehyde (MDA) activity. Superoxide dismutase (SOD) Lycopene supplementation of 10 mg/kg and 20 mg/kg showed a significant increase in SOD activity in comparison with the CMS group {F(6,35)}=25.87 [Table/Fig-4].

Glutathione Peroxidase (GPx)

Similarly, the activities of glutathione peroxidase (GPx) were found to be decreased significantly in the hippocampus of chronic mild stress group and vehicle treated group which is statistically significant {F(6,35)}=14.45; p<0.05, when compared to the control group. Lycopene supplementation dose of 10 mg/kg and 20 mg/kg improved the glutathione status of hippocampus when compared to supplementation of GPx levels in 5 mg/kg [Table/Fig-4].

Catalase (CAT)

There was a significant reduction in the CAT activity in the CMS group in comparison to the control group {F(6,35)}=18.71; p<0.05. Lycopene supplementation dose of 20 mg/kg and 10 mg/kg improved the Catalase status of the hippocampus {F(6,35)}=18.71.

Lipid Peroxidation

The activity of lipid peroxidation was found to be significantly higher in the hippocampus of rats exposed to CMS group and vehicle group in comparison to the control group which was statistically significant {F(6,35)}=17.14. Lycopene supplementation dose of 10 mg/kg and 20 mg/kg significantly reduced the lipid peroxidation {F(6,35)}=17.14 [Table/Fig-4].

Groups	SOD Units/mg protein	Catalase µmoles of H_2O_2 consumed/ min/mg protein	MDA (n moles of MDA formed/mg protein	GPX µ moles of glutathione oxidised/min/ mg protein period
Control	1.5±0.2	3.3±0.5	0.9±0.1	1.1±0.1
CMS (only stress)	0.5±0.8**	2.1±0.6***	1.9±0.1**	0.6±0.1**
CMS+vehicle	0.6±0.3**	1.3±0.4**	2.1±0.1**	0.5±0.1*
CMS+IMP 10 mg/kg	1.1±0.18##	3.3±0.3##	1.2±0.2##	1.1±0.2##
CMS+LYP 5 mg/kg	0.7±0.1	1.6±0.3	1.9±0.1	0.5±0.1
CMS+LYP 10 mg/kg	0.9±0.2##	3.0±0.4#	1.4±0.4#	0.9±0.1
CMS+LYP 20 mg/kg	1.1±0.09###	3.1±0.4##	1.2±0.2##	1.0±0.1##

[Table/Fig-4]: Effect of lycopene and imipramine treatments on antioxidant enzyme in control and CMS-treated rats.
The results are expressed as Mean±SD, (n=6) rats in each group. Control vs. CMS and CMS vs. lycopene (5, 10, 20 mg/kg). Statistical significance were determined by one way ANOVA followed by *Tukey's*, *p<0.05, **p<0.01, ***p<0.001 versus control and, #p<0.05, ##p<0.01, ###p<0.001 versus CMS

DISCUSSION

In biochemical investigations both serum corticosterone and brain, antioxidant enzyme activity was measured. Chronic mild stress, induces metabolic disturbances, by decreasing antioxidant enzymes activity in depressive patients [22]. The decrease in the antioxidant enzyme activity accounts for an increase in neuronal death, which remains a key factor in processing emotional disorders like depression and anxiety [23]. Chronic stress-induced disturbances in the HPA-axis often shows an increase in the serum cortiosterone levels and enlargement in the adrenal gland weight [24,25]. Lycopene dose (10 and 20 mg/kg) reversed the elevated serum corticosterone [Table/Fig-2].

The present findings also demonstrated an increase in the adrenal gland weight in CMS and vehicle rats compared with the non-stressed rats. Lycopene dose (10 and 20 mg/kg) reversed the hypertrophy of adrenal gland. An increase in the adrenal hypertrophy might be because of an increase in the release of Adrenal Corticotrophin Hormone (ACTH) released from the anterior pituitary gland [26,27] [Table/Fig-3].

Endogenous antioxidants play a major role in protecting cells against oxidative stress [28]. Increase in peroxidation of lipids causes a decrease in the brain antioxidant enzyme activity. Superoxide dismutase (SOD) catalase (CAT) and glutathione peroxidase (GPx) are the first line defense antioxidant enzymes [29]. SOD and CAT are the two important antioxidants in the brain whose activities were diminished during CMS [9,28]. This also shows a decrease in the SOD, CAT and GPX activity in the CMS group in comparison with the control. Increase in the levels of Malondialdehyde (MDA) in the CMS and vehicle groups were observed, indicating that rats are in a state of oxidative stress. The results of the present study, are in consistent with the previous reports [10,13]. A decrease in the SOD activity could be due to the activation of HPA-axis [30]. Lycopene supplementation significantly increased hippocampus SOD, GPx CAT activity and decrease in MDA activity levels. This shows antioxidant balance in the hippocampal region. Lycopene treatment significantly reverses this condition, of oxidative stress by increasing the enzyme activity. Lycopene showed antioxidant activity at a dose of 10 mg/kg and 20 mg/kg but failed to reverse these changes significantly at a dose of 5 mg/kg.

LIMITATION

The limitations of our study are, it is an experimental animal study with a small number of subjects. Hence, more studies with large number of subjects should be carried out to potentiate the results of this study.

CONCLUSION

The study concluded that chronic mild stress successfully induced oxidative stress in CMS and vehicle-treated groups, as it evidently increased the levels of MDA and decreasing Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione Peroxides (GPx) activity. Lycopene treatment successfully reverted oxidative stress by balancing the antioxidant enzyme activity and regulating the HPA-axis activity. Lycopene showed a protective effect, by up-regulating antioxidant enzyme activity.

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