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Lycopene Treatment Transposed Antidepressant-like Action in Rats Provoked to Chronic Mild Stress

P. Venkata Naveen Kumar*[1], P. Elango[1], S. Asmathulla[2] and S. Kavimani[3]

[1]Department of Pharmacology, Sri Ramachandra Institute of Higher Education And Research, Chennai, India.
[2]Department of Biochemistry, Sri Manakula Vinayagar Medical College And Hospital Pondicherry, India.
[3]Department of Pharmacology, Mother Theresa Post Graduate and Research Institute of Health Sciences, Puducherry, India.
*Corresponding author E-mail: drpelango@yahoo.com

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The present study aimed at evaluating the effects of lycopene on CMS-induced depressive-like behavioral changes in Wistar rats. In present study, rodents were selected randomly and grouped in to seven groups.Each group consists of six animals. All the groups are subjected to chronic mild stress in an unpredictable manner except the control group, which is free from stress. Behavioral changes induced during chronic mild stress were assessed by conducting the behavioral tests like forced swim test, sucrose preferences test, elevated plus maze test and open field tests in screening depressant and anxiety activity. The data analysis showed chronic mild stress produced depressive and anxiogenic behavior in the experimental rats. A significant increase in the immobility time and decrease in sucrose consumption in sucrose preference test are noted in CMS and vehicle groups. Similarly, in an elevated plus maze a significant decrease in the entries in the open arm and decrease in central square entries, and rearing behavior and increase in the duration of immobility were observed in open field test.Lycopene treatment for 6-weeks significantly decreased immobility time and increased in sucrose consumption observed in the forced swim test and sucrose preference test respectively. Lycopene significantly increased number of entries in the open arm of elevated plus maze and decreased grooming and freezing behavior in open field method. lycopene supplemented dose of 5mg/kg showed an insignificant results in all the behavioral models ($p>0.05$).The data were expressed as Mean±SD. Data were analyzed and differences between the means were determined by one-way analysis of variance (ANOVA) Using graph pad prism version 5.03 statistical software. In all the tests, differences were considered significant if $p<0.05$ to be a statistical significant. lycopene possesses antidepressant and mild- anxiolytic activity which may be due to its antioxidant effect that might warrant further studies.

Keywords: Chronic mild stress; Elevated plus maze test, Imipramine, Lycopene and Open field test.

Depression in common is a psychiatric disorder, which confers a fourth prominent cause of disability in worldwide by 2020 [1].Its prevalence in an aged group is considering, as an of the serious psychiatric disorder, affecting up to 20% of the world population. Characteristic features of marked depression are precisely mood and sleep disturbances, irritability with an apparent loss of pleasure and appetite[2]. Depression is a disorder with a multifactor etiology that includes in functional deficits in monoamines concentration and disturbance in HPA-axis in inducing functional and structural alterations in the brain[3,4].CMS model of depression is precisely

a valid model that inducing depression in rodents, and mimics in progress in the depressive state that had similar features in the human depressive state[5]. In this model, rats were inflicted to a variety of stressor over a considerable period of time, a gradual reduction in intake of sucrose and body weights with elevated serum corticosteroids that reflected by, a decrease in sensitivity to rewards namely(Anhedonia-loss of pleasure)is the most important symptom of depression[6].Decrease in the brain antioxidant enzyme activity and disturbances in HPA-axis is a major constraint factor, involved in processing emotional disorders like anxiety and depression[7].Dysregulation in HPA-axis is associated, with induction of depression [8].Chronic stress brings about structural changes in the hippocampus region that involved in regulating cognition[9].Conventionally used antidepressants increase reuptake of monoamines which crave 2-4 weeks to show antidepressant activity with 30% of remission. Currently used antidepressants shows more incidences of side effects like cognition impairment, sexual and sleep disturbances and precipitation of seizures. There is a considerable need in developing newer drugs with a lesser incident in potential side effects. Herbal drugs traditionally provide impressive scope in properly treating mood disorders with fewer adverse effects than synthetic compounds. Lycopene an antioxidant widely distributed in various fruits and vegetables [6]and highly lipid soluble and can readily cross the blood-brain barrier[10].Existences of a ß-ionic ring in its structure show it as a potent antioxidant. Lycopene well recorded as it exhibits conservation against cancer and cardio vascular disease [11].In according to Zhang et al., studied the antidepressant-like effect of lycopene in animal models by using lipid peroxidation for induction of depression[12]. In which author used a chemical method for induction of depression. In our study, we used the natural method for inducing depression and widely expected model in inducing depression. we hypothesized that CMS model induce stress in animals and bring behavioral changes in rodent, we anticipating lycopene supplementation could be potent molecule that could able to revert behavioral changes that are typically present during the induction of stress.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing between 240± 20 grams rats were housed 12hr light/day cycle, under controlled temp (22±2c) and humidity (40% -60%) with the standard chow feed and water were supplied at *ad libitum* throughout the procedure. (IAEC/52/ SRU/ 563/2017)(Sri Rama Chandra Institute of Higher Education and Research, Chennai India).All the test was conducted as per CPCSEA guidelines.

Drugs administration and CMS experimental design

Imipramine hydrochloride purchased from sigma-aldrich and lycopene powder is obtained parry nutraceuticals. Lycopene is lipid soluble and it's dissolved in corn oil which acts as a vehicle for a drug administered orally once daily for 6-weeks. Lycopene at doses of 5mg/kg,10mg/kg, 20mg/kg and standard drug imipramine at a dose of 10mg/kg.CMS and vehicle group received an equal volume of corn oil Control group will receive only saline water.

CMS Protocol

On completion of acclimatization rodents was divided into two groups control group and to-be -stressed group. A control group is placed in a separate room which is free from contact with other stressor groups. And to-be stressed group is subjected, to a serious of stressor in an unpredictable manner to prevent dependence. The stressor includes 1.Food and water deprivation-(A separate periods of food and water deprivation, and one period of both food and water deprivation.)2. Cage tilting- Tilt the cages backward (~ 45 degrees) 3.Soiled Cage -In which 250 ml plain water is poured into the sawdust. 4. Grouped housing, in which randomly changing the partners 5.Intermittent illumination -.Switch the room lights on and off every ~ 2hr)

Chronic mild stress

Wistar rats were transfer into the experimental room a week proceeding to initiate the experiment. Animals were fasted for 14 hours before conducting a consumption test rats are expose to a palatable 1% sucrose solution (100grams of sucrose dissolved in 100ml) for one hour at the end of the session bottles are

weighed, reduction in bottle weight gives an amount of sucrose consumed.CMS and vehicle groups are subjected to variety of stressor continuously for six weeks. Each stressor last for 10-14 hrs.

Behavioral Test

Sucrose preference Test(SPT)

Sucrose consumption test was performed as earlier described by[13]. In this test rodents were deprived of food and water 14hrs before the sucrose preference test. Wistar rats were trained to consume (1%) palatable sucrose solution and water in individual bottles *ad libitum* in each cage for a period of one hour.The consumption volumes of sucrose solution and water were recorded and calculated.[13].

Forced swim test(FST)

Forced swim test was conducted in an extensions glass cylinder of(height 40 cm and diameters of 20 cm,) filled with water maintained at a Temp (22-23°C) at a depth of 30 cm.FST activity was videotaped for 7 minutes. Initial 2 minutes of activity is discarded and the last 5 min of immobility was recorded and analyzed. Increase in duration of immobility in rats was regarded and more likely presented with depression-like state[14].

Open field test (OFT)

In open field test is used in screening, and assessing anxiety and exploratory behavior in rats[15].Exploratory behavior task was documented for five min and following activities were recorded a.)Ambulation behavior b) Rearing behavior number of times rodents stood on its hind legs, which measures locomotor activity and anxiety c)Grooming behavior rodents spend time itching itself while in standing position increased incidence of grooming is an indicator of anxiety [16]. The test was conducted in a semi -dark room of low light **(45 W fluorescent lights)** and apparatus painted with black floor divided into 16 squares of equal sizes and lines with the white colour of 6mm.

Elevated plus maze (EPM)

In elevated plus maze test is used in screening anxiety in rodents which show a naturally aversion to open spaces. It consists of two arms of opened and closed with dimensions of (45 ×10 ×0.5cm) and (45×10×30cm) respectively, positioned appositively to each other connected to a central platform of a dimension (10 ×10cm) and elevated 60 cm above the floor during a state of anxiety, rodents tend to move more towards the closed arm. Anti-anxiety treated rodents groups tend to move towards the open arm. Rats from each group were placed at the center of the maze, and activities were videotaped for 5 min activities like number of entries and duration of time spent in arms were recorded[16].

Statistical analysis

The results were expressed as Mean±S.D. Data were analyzed and differences between the means were determined by one-way ANOVA followed by *Tukey's multiple comparisons post hoc analysis*.Using graph pad prism version 5.03 statistical software. In all the tests, differences were considered significant if $p<0.05$, to be a statistical significant.

RESULTS

Sucrose preference test

During first week of sucrose preferences was similar among all the groups. At the end of 6-week there is a statistical significance differences in sucrose intake between groups were noted i.e. Control vs.CMS group.CMS group rats preferred less intake of sucrose than control group (i.e CMS 40% -50% and control group 60% to 62%) ($P<0.05$).Lycopene treated groups showed an increase in intake of sucrose in comparison with CMS.There was no statistically significant differences in sucrose consumption among lycopene treated group were noted**.**Figure:1

Forced swim test

In our study CMS group display a significant increased in immobility time in contrast with control group[$F(6,35)=10.08(p<0.01)$there was no significant variation in immobility time between lycopene supplementation at 5mg/kg and CMS group[$F(6,35)=10.08(p>0.05)$.More over, supplementation of lycopene at10mg/kg and 20mg/kg could able to reduce immobility time when compared to CMS group [$F(6,35)=10.08$ ($p<0.01$) in comparison to CMS group. Results are tabulated in.Table:2

Elevated plus maze

There is a significant difference in open arm entries between the control group versus CMS group[$F(6,35)= 6.865$],($p<0.05$) and similarly, in closed arm entries in control group versus CMS group [$F(6,35)=4.871$],($p<0.05$) respectively.

However, lycopene treatment at 5mg, could not able to show any statistical significance in comparison to the CMS group (p>0.005). Lycopene dose of 20mg/kg showed a significantly increased number of entries in open arm in comparison to the CMS group. Results are tabulated in Table: 3

Open field test

CMS group rats showed anxious behavior in comparison with the control group by decreasing central square entries which is statistical significant[F(6,35)=15.56] (**p<0.01**) and also showed a significant difference by increasing grooming [F(6,35)=8.770] and duration of immobility time in comparison with the control group (p<0.01).However, supplementation, of lycopene could significantly reverse these changes by decreasing the immobility period. lycopene at a dose of 20mg/kg could able to show anxiolytic activity. Results are tabulated in Table: 4

Table 1. Experimental Design

S.no	Experimental group	Dose	No. of animals	Treatment
1	Control	Saline	6	No Stress
2	CMS	Saline	6	Stress
3	CMS +Vehicle	Corn oil	6	Stress + vehicle
4	CMS +Impramine	10mg/kg bw-1	6	Stress + Treatment
5	CMS +Lycopene	5mg/kg bw-1	6	Stress + Treatment
6	CMS+Lycopene	10mg/kg bw-1	6	Stress + Treatment
7	CMS+Lycopene	20mg/kgbw-1	6	Stress + Treatment

Table 2. Effect of lycopene and imipramine treatments on forced swim test in control and CMS-treated rats

S.no	Groups	Duration of immobility in (sec)
1	Control	79.0±6
2	CMS	96.7 ±6**
3	CMS + vehicle	96.8±8*
4	CMS +IMP 10mg/kg	79.5±5 ##
5	CMS+LYP 5mg/kg	92.6±5
6	CMS+LYP10mg/kg	84 ±7 #
7	CMS+LYP 20 mg/kg	82 ±4 ##

Tables:2 Effect of lycopene on chronic mild stress induced rats subjected to forced swim test Data are expressed as Mean ± SD. (n=6) statistical significance were determined by one way ANOVA followed by *Tukey's*** p<0.01,*p< 0.05 versus control and ## p<0.01, # p< 0.05 versus CMS.

Tables:3 Effect of lycopene on chronic mild stress induced rats subjected to elevated plus maze test.Data are expressed as Mean ± SD.(n=6) statistical significance were determined by one way ANOVA followed by *Tukey's*** p<0.01,*p< 0.05

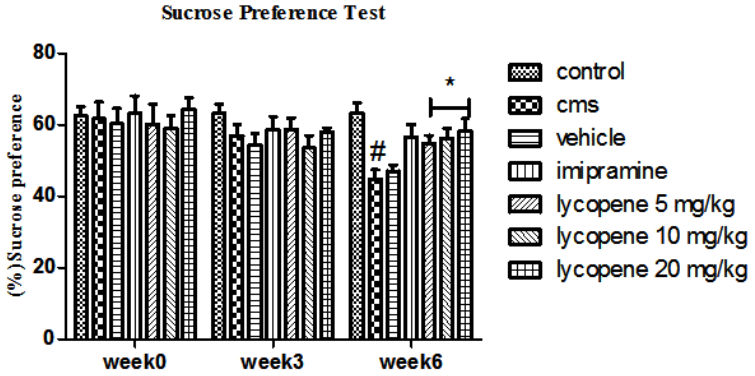

Fig. 1. Effect of lycopene and imipramine treatments on open field test in control and CMS-treated rats

versus control and ## p<0.01,# p< 0.05 versus CMS

Tables: 4 Effect of lycopene on chronic mild stress induced rats subjected to open field test. Data are expressed as Mean ± SD (n=6). Statistical significance were determined by one way ANOVA followed by *Tukey's*** p<0.01,*p< 0.05 versus control and ## p<0.01,# P< 0.05 versus CMS

DISCUSSION

Chronic mild stress method is the most valid model used in inducing depression and in studying progressive behavioral changes in rodents.[17]Chronic stress can provoke depression and anxiety in rodents [18].In this procedure, rodents are subjected to chronic mild stress for 6-weeks. Behavioral changes brought about during induction of stress,were studied by conducting behavioral tests like sucrose preference test, forced swim test and open field test, elevated plus maze test in rodents [19] .Lycopene is a potent antioxidant and has the highest antioxidant property among carotenoids.Lycopene doses were chosen in accordance with the previous studies (5mg/kg,10mg/kg and 20mg/kg)[20]. Rodents in a state of depressive-like behavior show more a state of immobility.This immobility state can be successfully reversible with antidepressant drugs [21]. In the present study, the CMS group showed an increase in the immobility time in comparison with the control group these results are consistent with the previous report [22,23].

Lycopene treated group showed an antidepressive like activity by decreasing immobility time in comparison with the CMS group. In sucrose preference test there is a low consumption of sucrose solution, which reflects the anhedonia symptom of depression.During stress, rodents show aversion towards consumption of sucrose those activities can be reversed by antidepressant treatment [24].CMS and vehicle groups showed a decrease in the consumption

Table 3. Effect of lycopene and imipramine treatments on elevated plus maze test in control and CMS-treated rats

S.no	Groups	Number of entries to Defecation	Open arm entry	Closed arm entry
1	Control	1.0±0.3	3.8±0.3	1.1±0.7
2	CMS	2.0±0.3	1.6±0.9*	3.1±0.7*
3	CMS + vehicle	2.2±0.2	1.8±1.0	2.8±0.7
4	CMS+IMP10mg/kg	1.1±0.2	3.6±0.9 #	1.6±1.2 #
5	CMS+LYP 5mg/kg	1.5±0.3	2.6±1.3	3.0±0.6
6	CMS+LYP10mg/kg	1.2±0.4	3.5±0.7#	1.6±1.0
7	CMS+LYP20 mg/kg	1.0±0.2	3.6±0.7 ##	1±0.6 #

Table 4. Effect of lycopene and lmipramine treatments on open field test in control and CMS-treated rats

Groups	Open Field Exploratory Tests Central square entries	Rearing	Grooming	Duration of immobility in sec
1.Control	5.5±1.3	8.3± 0.8	2.1±1.1	49.0±12.4
2.CMS	2.3±1.2**	4.1±2.0**	4.2±1.2*	92.8±18**
3.CMS + vehicle	1.8±1.1 **	4.1±2.4*	4.6±1.3*	109.0±27*
4.CMS+IMP 10mg/kg	8.0±1.7##	8.8±0.7 ##	2.0±1.6##	55.1±13##
5.CMS+LYP 5mg/kg	3.3±1.6	5.6±2.4	4.8±0.7	110±22.4
6.CMS+LYP10mg/kg	3.0± 1.5	6.1±2.7	2.3±1.0	74±18.0
7.CMS+LYP20 mg/kg	4.8±1.6#	8±1.6#	1.8±1.1##	55±9.9 #

of sucrose which is reverted by lycopene treatment.Behavioral models like elevated plus maze and open field tests are commonly used in screening anxiety in rodents.Variables that can be determined during elevated plus maze that includes a number of entries in each arm(i.e. open arms and closed arms)[25]and also used in assessing the anti-anxiety mechanism of action [26,27].Previous studies are supporting the pharmacological validation of EPM with the use of anxiolytics like benzodiazepines,the effect of anxiolytic drugs is to cause an increase in the number of crossings onto the open arms of EPM and consequently reduce time spent in the closed arms of EPM. Lycopene supplemented groups showed a decrease in a number of entries in the closed arm and an increased number of entries in the open arms. **Table:3**. Therefore, lycopene may be considered as an anxiolytic agent. It showed significant results at a dose of 20mg/kg.

Open field test is used to study exploratory and anxiety behavior in rodents. Whose actives are diminished during chronic mild stress. Variables that can be determined in open field method like rearing, grooming, immobility time and central square entries. Increase in grooming and decrease in central square entries are the indicators of anxiety[28].Grooming rodents spend time itching itself while in standing position increased grooming and immobility time is an indicator of anxiety.Anxiolytic drugs like diazepam increase exploratory behavior,where as anxiogenic drugs like picrotoxin decrease exploratory activity. Lycopene supplementation dose of 20mg/kg showed a significant number of entries into the central square versus the CMS group.Showed lycopene protection against the exploratory behavioral changes induced during induction of stress.Table:4.These results are comparable to the standard drug imipramine at a dose of 20 mg/kg.

Rearing the number of times rodents stood on its hind legs, a decrease in rearing activity shows a reduction in exploratory activity[28].CMS group should a decreased rearing behavior in comparison with the control group.lycopene supplementation showed increased rearing behavior at a dose of 20mg/kg.

According to previous literature lycopene showed the protective effect in regulating depressive-like behavior induced by lipopolysaccharide, a chemical which produces neuro-inflammation in brain[29].However,in this study, there is a lack of standard screening method for inducing depression. Lycopene supplemented dose of 5mg/kg could not show, much alteration in any of behavioral models. Lycopene at a dose of 10mg/kg showed a moderate response in forced swim test and elevated plus maze test, but did not show any significant response in open field method. Our data concluded that lycopene supplementation dose of 20mg/kg has,anti-depressive activity and moderate anxiolytic activity.The standard drug imipramine showed antidepressant activity and anxiolytic activity by increasing the proportionate number of entries in the open arm in the EPM test. Similarly, the duration of mobility and preference in sucrose consumption are increased in FST and SPT respectively indicates that imipramine reversed stress-induced behavioral alterations.

During lycopene supplementation of 6-weeks, there is no adverse effects were noted in rodents. Since lycopene doses were selected at a minimum dose.It was proved that lycopene dose of (3g/kg.d)to be the safest dose in rodents[30].These study findings have concluded that multifactorial event in the brain during the induction of stress, which might progress into neurodegenerative changes in the brain that manifested in the behavioral model. The strength of this study is that use of the most valid method in inducing depression.

CONCLUSION

Study protocol, could successfully adeptly induce depression and anxiety-like activity in rodents. Lycopene treatment could able to successfully reverted this condition because of its antioxidant activity and lipid soluble in nature, which can readily cross the blood -brain barrier which might improve the CNS functions. Further studies may be needed to elucidate the possibility of its mechanism of action.

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