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Original Research Article

Evaluation of sesamol for anxiolytic activity in 72 hours sleep deprivation model in C57BL/6 mice

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ABSTRACT

Background: In our study, we explored anxiolytic activity of sesamol in C57BL/6 mice. Anxiety was induced in mice after 72 hours of sleep deprivation. Anxiety was assessed by three behavioural tests as Open field test, hole board test and light dark chamber test.
Methods: In our study, sesamol was evaluated in C57BL/6 mice for anxiolytic activity. Anxiety was induced by flower pot technique, in which sleep deprivation was done in mice for 72 hours. After 72 hours of sleep deprivation, the animals were subjected to three behavioural tests, as open field test, Hole board test and Light dark chamber test. In the Open field test, parameter assessed was time spent in central square, that was considered as a measure of anxiolytic activity. In hole board test, increase in number of head dips was indicative of anxiolytic activity. Anxiety assessment was also done by light dark chamber test, where more time spent in light chamber is considered as indicative of anxiolytic activity.
Results: In open field test, sesamol 20 mg/kg group, the time spent in central square was statistically significant ($p<0.05$) in post hoc comparison, suggesting that sesamol exhibited anxiolytic effect in animals. In hole board test, sesamol 20 mg/kg group showed significant increase ($p<0.05$) in number of head dips indicating its anxiolytic activity in the said model. In light dark chamber test, there is significant increase in anxiolytic activity in diazepam group as compared to control group, while not significant with sesamol in either dose in light dark chamber test.
Conclusions: So, to conclude, sesamol exhibited statistically significant anxiolytic activity in the dose of 20 mg/kg in open field test and hole board test.

Keywords: Sesamol, C57BL/6 mice, 72 hours sleep deprivation, Anxiolytic activity

INTRODUCTION

Most common mental disorders are anxiety disorders, which include panic disorder/agoraphobia (PDA), generalised anxiety disorder (GAD), social anxiety disorder (SAD), and others**.** Nonpharmacological psychotherapy, medication, or a combination of the two can be used to effectively treat anxiety disorders.[1] Among the pharmacological strategies for anxiety are Selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors are the first-line medications (SNRIs) benzodiazepines (BZD) and azapirones, sedative anti histaminics and beta blocker. Regular usage of benzodiazepines is not advised owing to the possibility of addiction. Other medication options are Pregabalin, a calcium modulator, tricyclic antidepressants, buspirone, moclobemide, and other medications are further therapy of choices. Buspirone do not have disadvantage of benzodiazepines. These treatment strategies cause various adverse effects such as dependence, cognitive dysfunction, muscle weakness, dizziness, weight gain, insomnia, loss of appetite, blurry vision, feeling agitated, weight gain, difficult urination.[2]

Non-pharmacological treatments are an integral part of management in psychiatric illnesses, which includes cognitive behavioural therapy, deep brain stimulation, electroconvulsive therapy, transcranial magnetic stimulation, vagal nerve stimulation, magnetic seizure therapy, physical exercise and lifestyle changes.[3]

The undesirable side-effects associated with the pharmacological compounds, used in conventional medicine, support the relevance for search for alternative therapies with higher efficacy and bioavailability, as well as with fewer side-effects.[4] In this context, plants can be a veritable source of novel compounds with therapeutic value for psychiatric disorders. This also supports a continued need for search of newer or alternative strategies, to find better therapeutic agents with better efficacy and safety profile. In this context, plant-based products have also been explored as *Curcuma zanthorrhiza, Withania somnifera, Rauwolfia serpentina, Datura, convolvulus pluricaulis, Commiphora whighitti,* etc for their neuroprotective activity.[5] Hence, plant products are increasingly been explored as extracts-ethanolic/methanolic/hydroalcoholic or devised as other herbal formulations as per our literature search. So, the present scenario witnesses the surge of natural products or herbal medicines that have received considerable attention and has led to many upcoming strategies for various ailments as well as newer drug discoveries and development.[6] Hence, we have explored sesamol, a phytoconstituent obtained from sesame seeds, for anxiolytic activity. Till date antianxiety effect of sesamol found to be due to oxidative stress.

METHODS

The animals were used from central animal facility of AIIMS Bhopal and all the procedure were conducted inside the same facility. The study was conducted after obtaining approval (IAEC-034) from the institutional animal ethics committee (IAEC), AIIMS Bhopal. In this study total 90 mice (C57BL/6 strain, 25-30 gm) were used. The animals were maintained under standard laboratory conditions with natural dark and light cycle. Animals were allowed free access to dry rodent diet and tap water ad libitum. All efforts were made to minimize animal suffering and to use only the number of animals to produce reliable scientific data.

Drugs were purchased from authorized commercial supplier. Sesamol (Sigma Aldrich Pvt Ltd India), diazepam (Medibios laboratories Pvt Ltd India). Drugs were freshly prepared before administration, and dosing done as per protocol. Normal saline or DMSO was used as vehicle. Doses of drugs were used according to previously done studies.

Sesamol was given for 7 days in the doses as per protocol.[7] Sleep deprivation was induced by flower pot technique, were in mice were deprived for 72 hours, the mice were placed on a single small platform in a bucket of water.[8,9]

Table 1: The groups were as follows.

Groups	Interventions	Number of mice
1.	Vehicle	6
2.	Sleep deprived mice (72 hrs)	6
3.	Sesamol (10 mg/kg p.o) + sleep deprivation (72 hrs)	6
4.	Sesamol (20 mg/kg p.o) + sleep deprivation (72 hrs)	6
5.	Diazepam (2 mg/kg p.o) + sleep deprivation (72 hrs)	6

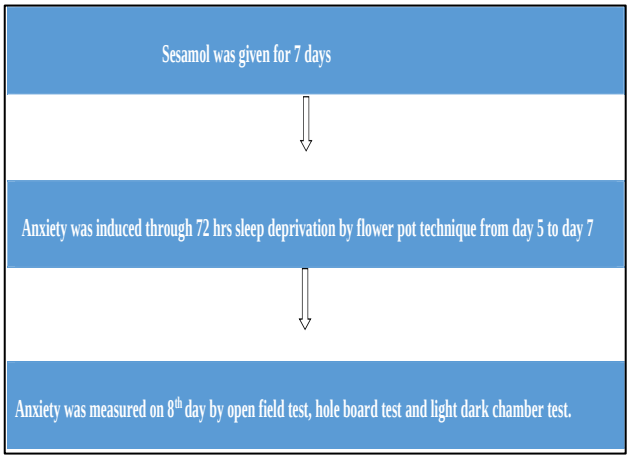

Figure 1: The protocol to assess antianxiety activity was followed as described below.

Behavioural tests for anxiety measurement

We have used battery of test to measure of anxiety behaviour, as three validated models, open field test, hole board test and light dark box test in single setting for each animal.

Open field test

During this test, mice were allowed to move and explore the open field box (60×60 cm) that consisted of 16 equal squares, for a period of 5 minutes, and recorded by video camera. Total time spent in central square was calculated as measure of anxiolytic behaviour.[10] The box was cleaned with 95% alcohol after every test.

Parameter assessed were total time (in secs) spent in central square.

Hole board test

The apparatus consists of a box (40×40×25 cm) with 16 evenly spaced holes (3 cm). Each mouse was left in the centre and allowed to freely explore. Number of head dips into the holes was recorded for a period for 5 minutes, increase in number of head dips was considered as anxiolytic behaviour.[11]The board cleaned with 95% alcohol after every test and allow to air dry.

Parameter assessed number of head dips.

Light-dark chamber test

The light-dark chamber (45×20×25 cm) is made up of a light chamber (30×20) and a separate dark chamber (15×20 cm) with a single opening (5×4) for passage in between them. The total time spent in the light chamber was recorded by video camera for 5 min and considered as measure of anxiolytic behaviour. A mouse is defined to have entered the light and dark chamber when both shoulders and front paws are inside the respective compartment.[12]The apparatus was cleaned with 95% alcohol after every test to avoid the left -over effects.

Parameters assessed were time (sec) spent in light chamber.

The quantitative and continuous data obtained from the all-study groups was analysed using statistical package, SPSS IBM ver 26.0. Data was presented as mean ± SD. Analysis of variance (ANNOVA) was used to compare the outcomes in intergroup data and control groups. Post -hoc analysis of study was done by using 2 tailed Dunnet`s test. Probability value less than 0.05 ($p<0.05$) taking 95% confidence interval (CI) was considered for testing statistically significance.

RESULTS

Anxiety was induced by flower pot technique, and later on assessed for antianxiety activity by battery of tests using open field test, hole board test and light-dark chamber tests were done. All groups were compared with control group. In Open field test time spent in central square was tested. In hole board test number of head dips were tested and in light dark chamber test the time spent in light box was tested.

In open field test, time spent in central square of all groups was compare with control group. There was significant increase in time spent in central square in sesamol 20 mg/kg and diazepam group as compared to control group. Time spent in central square by sesamol 20 mg/kg group is comparable to diazepam group (Table 2 and Figure 2).

In hole board test, mean number of head dips of all groups were compared with control group. There was significantly increase in number of head dips in sesamol 20 mg/kg and diazepam group as compared to control group. Number of head dips significantly increase in sesamol 20 mg/kg and diazepam group, (Table 3 and Figure 3).

In light dark chamber test, mean time spent in light box of all groups were compared with control group. There was significantly increase in mean time spent in light box in diazepam group as compared to control group but there was significant decrease in time spent in light box in sleep deprivation group (Table 3).

Table 2: Mean difference in time spent in central square among different study groups in open field test.

Study groups	Treatment	Mean ± SD (Time spent in central square)	P value*
Control	DMSO	16.33±14.47	-
Sleep deprivation	Sleep deprivation 72 hrs	5.67±2.37	0.572
Sesamol 10 mg/kg	Sesamol (10 mg/kg p.o) + sleep deprivation 72 hrs	14.00±10.69	0.997
Sesamol 20 mg/kg	Sesamol (20 mg/kg p.o) + sleep deprivation 72 hrs	39.67±24.13	0.048*
Diazepam	Diazepam (2 mg/kg p.o) + sleep deprivation 72 hrs	42.50±16.05	0.047*

P* value one way ANOVA followed by 2 tailed Dunnet`s test. *Mean time spent in central square significantly increased in sesamol 20 mg/kg group. **Mean time spent in central square significantly increased in diazepam group.

Table 3: Mean difference in number of head dips among different study group in Hole board test.

Study groups	Treatment	Mean ± SD (Number of head dips)	P value*
Control	DMSO	14.17±9.60	-
Sleep deprivation	Sleep deprivation 72 hrs	10.17±5.07	0.971
Sesamol 10 mg/kg	Sesamol (10 mg/kg p.o) + sleep deprivation 72 hrs	29.67±21.01	0.236
Sesamol 20 mg/kg	Sesamol (20 mg/kg p.o) + sleep deprivation 72 hrs	36.67±12.78	0.032*
Diazepam	Diazepam (2 mg/kg p.o) + sleep deprivation 72 hrs	42.25±15.50	0.015*

*P value one way ANOVA followed by 2 tailed Dunnet`s test. *Mean number of head dips significantly increased in sesamol 20mg/kg group. **Mean number of head dips significantly increased in diazepam group.

Table 4: Mean difference in time spent (in secs) in light chamber among different study groups in light dark-chamber test.

Study groups	Treatment	Mean ± SD (Time spent in light box)	P value*
Control	DMSO	159.17±17.40	-
Sleep deprivation	Sleep deprivation 72 hrs	76.83±18.67	0.001*
Sesamol 10 mg/kg	Sesamol (10 mg/kg p.o) + sleep deprivation 72 hrs	150.67±28.59	0.976
Sesamol 20 mg/kg	Sesamol (20 mg/kg p.o) + sleep deprivation 72 hrs	191.83±57.76	0.278
Diazepam	Diazepam (2 mg/kg p.o)+ sleep deprivation 72 hrs	219.75±13.91	0.030*

P* value ANOVA followed by 2 tailed Dunnet's test. *Mean time spent in light box significantly decreased in sleep deprivation group. **Mean time spent in light box significantly increased in diazepam group.

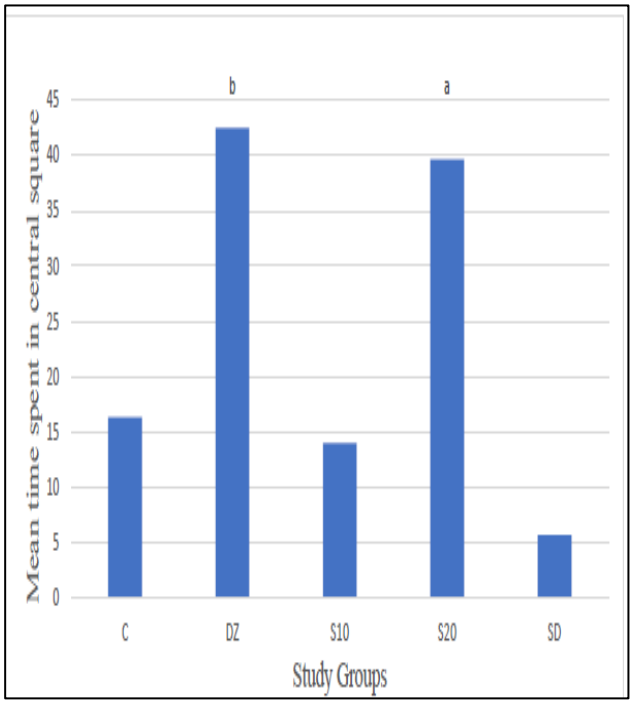

Figure 2: Mean time spent in central square (secs) among the different study groups in open field test.

All values are mean (n=6). [a]p<0.05, [b]p<0.05(one way ANOVA followed by Dunnet's 2 tailed test) C=control, DZ=diazepam, S10=sesamol 10 mg/kg, sesamol 20 mg/kg, SD=sleep deprivation.

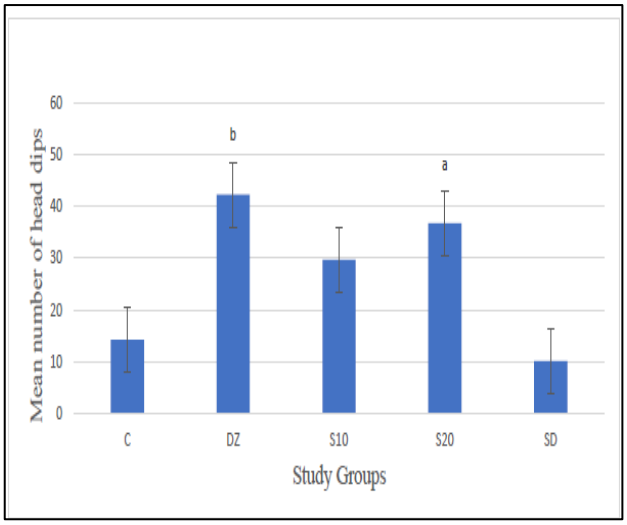

Figure 3: Bar graph showing number of head dips among different study groups.

All values are mean (n=6). [a]p<0.05, [b]p<0.05(one way ANOVA followed by Dunnet`s 2 tailed test) C=control, DZ=diazepam, S10=sesamol 10 mg/kg, sesamol 20 mg/kg, SD=sleep deprivation.

Figure 4: Flower pot technique to induce anxiety in mice.

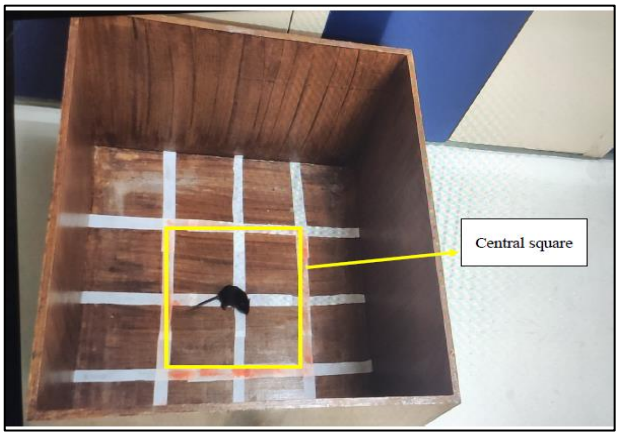

Figure 5: Mouse exploring central square in open field test.

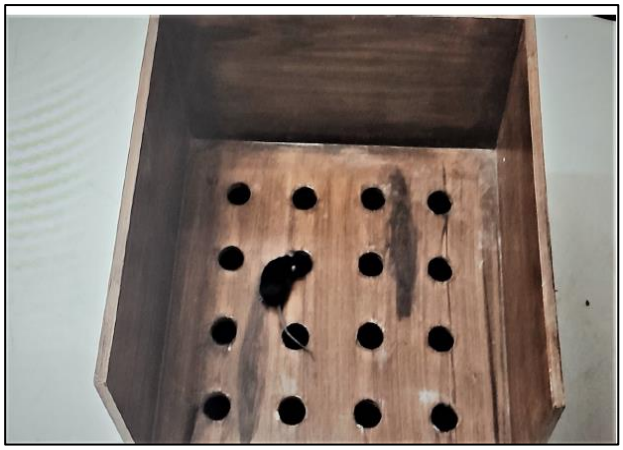

Figure 6: Mouse exploring hole board apparatus in Hole board test.

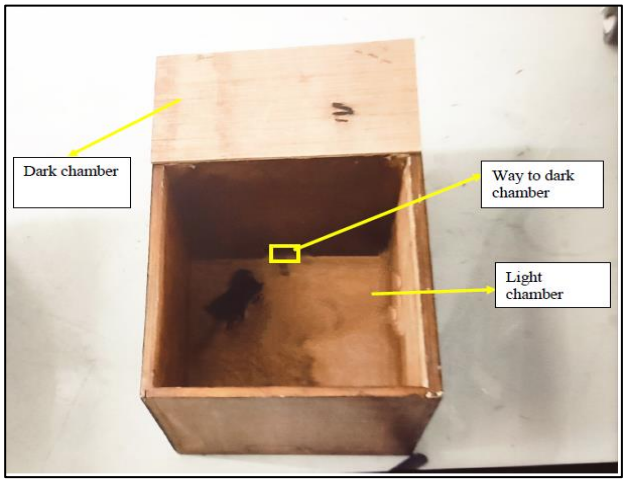

Figure 7: Mouse exploring light box in light dark chamber test.

DISCUSSION

In our study, anxiety was assessed by three behavioural tests as open field test, hole board test and light dark chamber test as well. Anxiety was induced by flower pot technique, in which sleep deprivation was done in mice for 72 hours. The mice were placed on single small platform in a bucket of water in this technique, such that the animal could not go in rapid eye movement (REM) sleep which leads to increase in oxidative stress in animals. After 72 hours, animal was subjected to behavioural tests.[10-12] In open field test, parameter assessed was time spent in central square is considered a measure of anxiolytic activity, less anxious animal spends more time in central square.[10]

In our study, in the open field test, the time spent in central square was compared and it was observed that there was significant increase in time spent in central square in between groups. Time spent in central square by positive control group (diazepam group) in the study was more as compared to control group, indicating lesser anxiety in these animals. In sesamol 20 mg /kg group, the time spent in the central square was statistically significant ($p<0.05$) in post hoc comparison, suggesting that sesamol exhibits anxiolytic effect in these animals.

The number of head dips were compared among different study groups in hole board test, the increase in the number of head dips is indicative of anxiolytic activity.[10] There was significant increase in number of head dips in between groups suggestive of decrease in anxiety. In post hoc comparison, sesamol 20 mg group showed significant ($p<0.05$) increase in number of head dips indicating it`s anxiolytic activity. Studies are needed to further explore sesamol as an anti-anxiety agent or as anxiolytic-like agent/drugs.[14,15] Anxiety assessment was also done by light dark chamber test, time spent in light chamber was compared among different groups; more time spent in light chamber is indicative of anxiolytic activity.[12] The time spent in light chamber was significantly decreased by sleep deprived group as compared to control group. In post hoc comparison, there is significant increase in anxiolytic activity in diazepam group as compared to control group, while not significant with sesamol in either-doses.

Previous study concluded for significant anxiolysis where combination of sesamol and buspirone potentiated the antianxiety effects in immobilisation stress model. Pre-treatment with the combination, improved body weight, locomotor activity as well as anxiety- like behaviour in this test.[14] So, overall results of post hoc analysis for antianxiety activity in our study showed statistically significant anxiolysis exhibited by sesamol at 20 mg/kg dose in open field and hole board test, while not significant for light-dark chamber test. Further study are needed to explore sesamol and whether it can find a place as a strategy in the category of anxiolytic-like activity (ALA) drugs.[13,14]

Limitations

In our study we have recorded all the data by video recording and no automated system or software has been used in our study. However, to minimize the bias, interpretations of blind observer were also taken in account for each recording. All calculations in our study were done manually, data was re-analysed with the help of blind observer. Biochemical test may be improvised to identify additional components related to activities explored in our study.

CONCLUSION

We found significant results of sesamol in a dose of 20 mg/kg in open field and hole board behavioural tests in assessing anxiolytic activity as compare to control group. The improvement in open field test suggestive of anxiolytic activity as the time spent by the animal is increased in the central square. Also, anxiolytic activity was evident in hole board test as there was increased head dips for sesamol 20 mg/kg dose. Hence, our study results

suggestive of anxiolytic activity of sesamol in a dose of 20 mg/kg.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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