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Evaluation of Anxiolytic Activity of Sesamol in Swiss Albino Mice

Sriram Chandra Shaker¹, Lahkar Mangala², Kasala Eshvendar Reddy^{1*}

1. Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER), GMC IIIrd Floor, Bangarh, Guwahati- 781032, India.

2. Department of Pharmacology, Gauhati Medical College, Bangarh, Guwahati-781032, India

ABSTRACT

In the present study, effects of increasing doses of sesamol (25, 50 and 100mg/kg) on anxiety were investigated in adult Swiss albino mice using elevated plus-maze, light/dark box, open field and hole board tests as animal models compared with the effects of the standard anxiolytic diazepam (1.0 mg/kg) and vehicle, which were administered intraperitoneally. Sesamol was found to significantly increase the time spent and number of entries in open arm when compared to closed arm in elevated plus maze apparatus. In light/ dark box model, time spent in light box and number of crossings between compartments increased significantly. In open field test, significant increase in number of rearing, assisted rearing and number of square crossed were observed. It was also observed that sesamol significantly increased the number of head dips in hole board test. These results infer that, sesamol having anxiolytic activity in a dose-dependent manner.

Keywords: Anxiolytic activity, Sesamol, Diazepam, Elevated plus-maze, Light/dark box, Open field, Hole board.

*Corresponding Author Email: ishreddy4@gmail.com

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INTRODUCTION

Anxiety disorders are among the most common of psychiatric disorders. This contributes to a significant public health burden. Excessive anxiety can debilitate and damage the quality of life¹. The prevalence of these disorders is increasing in many countries and have a much earlier age of onset than other chronic conditions². Anxiety affects one of every eight persons worldwide and has evolved interest in the area of research concerned with psychopharmacology during this decade³. In the clinical treatment of anxiety benzodiazepines, GABA-A receptor agonist and buspirone, a 5-HT_{1A} receptor agonist, are mainly prescribed as first choice treatment. However chronic administration of benzodiazepines, results in physical dependence such as sedation, myelorelaxation, ataxia, amnesia and pharmacological dependence⁴. Furthermore buspirone also results in dizziness, headache, nervousness, paresthesia, diarrhea, excitation and sweating as adverse effects⁵. Therefore, research has to be conducted to identify safer, more specific medications possessing anxiolytic effect without these complications. The study of medicinal plants and plant derived products could provide new therapeutic options⁶. In past few years, search for novel pharmacotherapy from medicinal plants for psychiatric disorders has progressed significantly and this has been reflected in many studies⁷.

Sesame, an important oilseed from *Sesamum indicum* (Family: Pedaliaceae), is one of the oldest oilseeds known to man and is considered to have not only nutritional value, but also some medicinal effects. It has been employed in ancient Chinese medicine to prevent aging⁸. Traditionally, sesame seed paste (upon topical application) and its oil (orally and topically) have been used for promoting healing in burns and other wounds⁹. The oil is reported to have the following constituents – sesamol, sesamolin and sesaminol¹⁰. Sesamol is a phenolic derivative with a methylenedioxy group that is a component of sesame seed oil. Studies suggest that sesamol possesses antioxidant¹¹, photo-protective¹², chemopreventive¹³ and anti-mutagenic¹⁴ effects. A recent study revealed that chronic administration of sesamol potentiates anti anxiety and antioxidant like effect of buspirone in sleep loss induced anxiety model in mice¹⁵. Till date, there is no such report available on the sesamol in acute animal models of anxiety in mice. On the basis of these considerations, it was the purpose of this study to characterize the anxiolytic activity of sesamol.

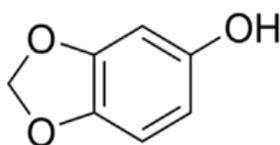


Figure 1: Structure of Sesamol

MATERIALS AND METHODS

Animals:

Adult male Swiss albino mice (18–24 g) were procured from Pasture institute, Shillong, India, kept in polypropylene boxes covered with sawdust and maintained at 25 ± 1 °C, $55\pm 5\%$ humidity along with 12 h light/dark cycle (lights on at 7 a.m.). The animals were given standard pellet diet and water ad libitum throughout the experimental period except 12 h before and during the experiments. The experiments were conducted according to the Institutional Animal Ethics Committee (Guahati medical college) regulations approved by the Committee for the purpose of Control and Supervision of Experiments on Animals.

Experimental design:

Animals were divided into five groups, each containing six mice. Group I served as vehicle control and received 0.9% (w/v) of saline (1 ml/100 g). Group II served as positive control and received diazepam (1 mg/kg). Group III, Group IV and Group V were considered as test groups and received 25, 50 and 100 mg/kg of sesamol respectively. All treatments were administered intraperitoneally (i.p) 30 min prior to start of the experiment.

Drugs and treatments

Sesamol procured from Sigma Aldrich was dissolved in distilled water immediately before use and was administered intraperitoneally, at increasing doses (25, 50 and 100mg/kg) at a volume of 0.1 mL/10g body weight. Diazepam (Ranbaxy, India) was dissolved in distilled water and used as a positive control (1 mg/kg), administered intraperitoneally at a volume of 0.1 mL/10 g body weight. Vehicle control group were given intraperitoneal injections of saline.

PHARMACOLOGICAL ASSAYS

All the behavioral tests were carried out between 09:00 a.m. and 02:00 p.m. in undisturbed and temperature controlled room (25 ± 2 °C), illuminated with a 40W fluorescent bulb. The animals were fasted 12 h prior to the experiment. 1 h before the administration of the drugs, mice were moved into the laboratory to habituate to the experimental environment. Each animal was used only once.

Elevated plus maze test

Elevated plus maze (EPM) consists of two open arms (35x5 cm) crossed with two closed arms (35x5x20 cm). The arms were connected together with a central square of 5x5 cm. The apparatus was elevated to the height of 25 cm in a dimly illuminated room. Sesamol (25, 50 and 100 mg/kg), Diazepam (1mg/kg) and vehicle were administered to the mice before being placed

individually in the centre of the EPM, facing a closed arm. The number of entries and time spent in both the open and closed arms were recorded for 5 min. An entry was defined as having all four paws within the arm.

Open field test

The open field test was used to evaluate the exploratory activity and emotional response of the animals. The apparatus consisted of a wooden box (60x60x30 cm). The base of the box was divided into 16 squares (15x15 cm). The apparatus was illuminated with 40-W lamp suspended 100 cm above it. Mice were treated with sesamol (25, 50 and 100 mg/kg), vehicle and diazepam. After 30 min they were placed in one of the corner squares, the number of rearing, assisted rearing (forepaws touching the walls of the apparatus) and the number of squares of crossed were counted for 5 min.

Light/dark exploration test

This test is based on the innate aversion of rodents to brightly lit areas and on their spontaneous exploratory behavior in response to a novel environment and to light. The apparatus consisted of two boxes (25x25x25 cm) joined together. One box was made dark by covering its top with plywood, whereas a 40-W lamp illuminated the other box. The light source was placed 25 cm above the open box. The mice were placed individually in the center of the lit box and observed for the next 5 min for the time spent in the lit and dark boxes. The mice were treated with sesamol (25, 50 and 100 mg/kg), diazepam and vehicle, 30min before being placed in the lit box.

Hole board test

The hole board apparatus consisted of a wooden box (40x40x25 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the base of box. The apparatus was elevated to the height of 25 cm. Mice were given sesamol (25, 50 and 100 mg/kg) diazepam and vehicle, 30 min before they were placed in the apparatus. The number of head pokes during a 5 min period was recorded.

Statistical analysis

All the results were expressed as mean± S.E.M. obtained by groups of 6 animals each. Differences between experimental groups were analyzed by one-way ANOVA followed by Dunnett's post-hoc test using GraphPad Prism Software (version 5) with the experimental groups being compared with the vehicle control. Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION:

Elevated plus maze model

The effect of treatments on the behavior of mice in EPM was tabulated in Table 1. Results shows that both sesamol (25, 50 and 100mg/kg) and diazepam resulted in an increase in the time spent on the open arms ($p < 0.05$, $p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively) and the number of open arm entries was significant ($p < 0.01$, $p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively) compared to the vehicle control group. Administration of sesamol at increasing doses (25, 50 and 100mg/kg) has resulted in both decreased time spent and entries into the closed arm significantly in a dose dependent manner. This behavior in EPM is an indicative of anxiolytic activity¹⁶ and is similar to the positive pattern effects expected following the use of anxiolytics drugs like diazepam^{17, 18}.

Table 1: Effect of different concentrations of sesamol and diazepam on the time spent on open & closed arms and on the number of entries on open & closed arms in EPM test in mice.

Treatments	Time spent (in sec) (Mean \pm SEM)		No .of entries (Mean \pm SEM)	
	Open arm	Closed arm	Open arm	Closed arm
Vehicle control	40.23 \pm 1.12	112.52 \pm 2.26	4.27 \pm 0.36	17.30 \pm 1.13
Diazepam (1mg/kg)	76.5 \pm 1.33***	82.76 \pm 1.2***	10.45 \pm 0.52***	7.8 \pm 1.24***
Sesamol (25 mg/kg)	45.67 \pm 1.42*	105.38 \pm 1.24*	6.28 \pm 0.43**	12.40 \pm 1.4*
Sesamol (50mg/kg)	58.43 \pm 1.26***	101.62 \pm 1.86**	7.37 \pm 0.47***	10.13 \pm 1.1**
Sesamol (100mg/kg)	71.35 \pm 1.72***	87.23 \pm 1.82***	8.74 \pm 0.38***	8.74 \pm 0.94***

All the values are expressed as mean \pm SEM (n = 6); *P < 0.05; **P < 0.01; ***P < 0.001 Vs vehicle control.

Light/dark box test

The results of the light/dark box test are shown in Figure 2-4. As shown in Figure 2, sesamol (50 and 100mg/kg) and diazepam group were spent significantly more time in the brightly lit chamber when compared to vehicle control group ($p < 0.01$, $p < 0.001$ and $p < 0.001$). But only animals treated with highest dose of sesamol and diazepam (1mg/kg) were significantly decreased time spent in the darkly lit area when compared to vehicle control ($p < 0.001$) (Figure 3). In addition, there was a significant increase in the number of crossings between the compartments as a consequence of the effect of sesamol (50 and 100mg/kg) and diazepam as compared to vehicle control ($p < 0.001$) (Figure 4). The results were consistent with data in the literature^{19,20}, showing an increase in the time spent in the brightly lit compartment (Figure 2) and an increase in the number of movements from one compartment to the other (Figure 4) confirming its role as a positive control of anxiety.

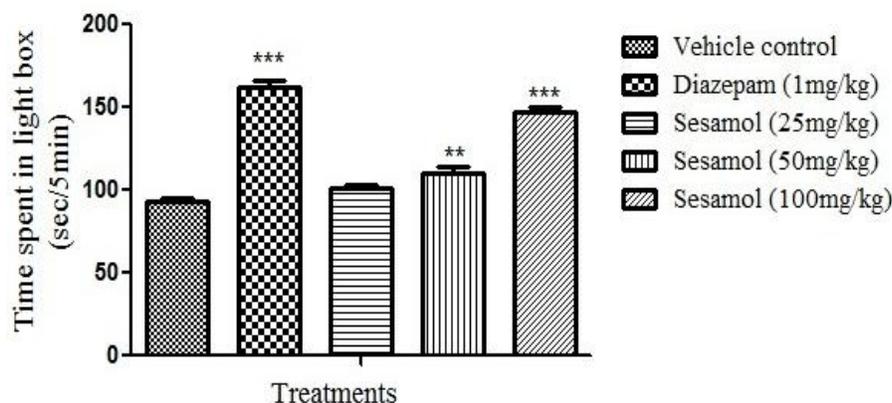


Figure 2: Effect of sesamol on time spent in light box in light/box exploration test over a 5 min period of time. All the values are expressed as mean \pm SEM (n = 6); *P < 0.05; **P < 0.01; ***P < 0.001, compared with vehicle control.

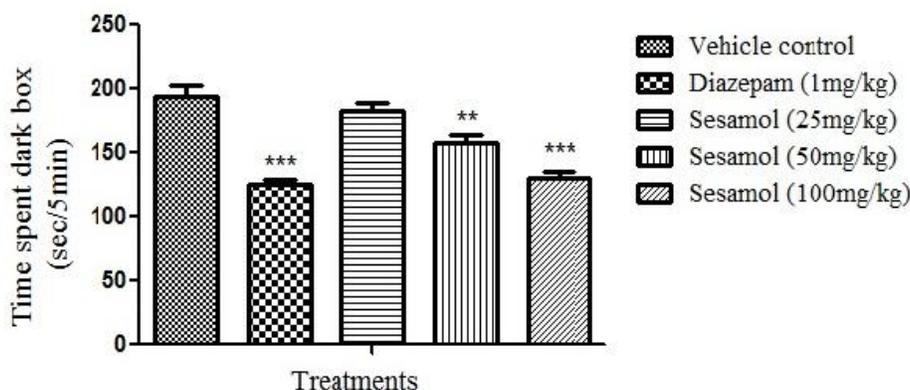


Figure 3: Effect of sesamol on time spent in dark box in light/box exploration test over a 5 min period of time. All the values are expressed as mean \pm SEM (n = 6); *P < 0.05; **P < 0.01; ***P < 0.001, compared with vehicle control.

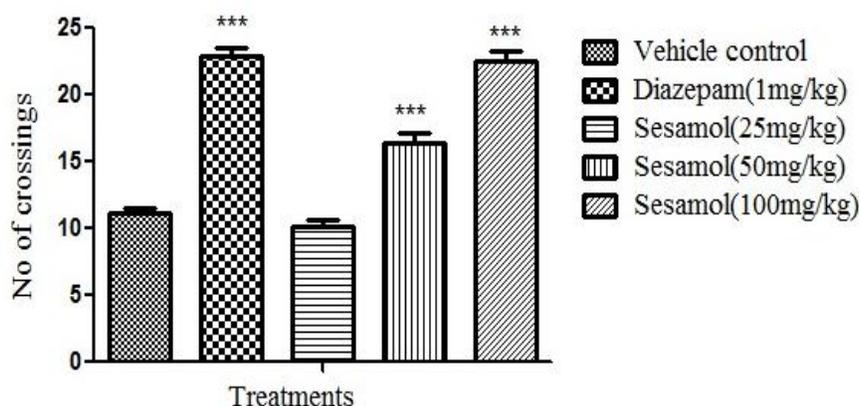


Figure 4: Effect of sesamol on transition number between compartments in light/box exploration test over a 5 min period of time. All the values are expressed as mean \pm SEM (n = 6); *P < 0.05; ** P < 0.01; *** P < 0.001, compared with vehicle control.

Open field test:

As shown in Figure 5 and 6, sesamol (25, 50 and 100mg/kg) and diazepam groups increased the number of rearing ($p < 0.01$, $p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively) and assisted rearing ($p < 0.05$, $p < 0.001$, $p < 0.001$ and $p < 0.001$, respectively) as compared to vehicle control group. In this experiment sesamol, significantly increased the number of self rearing (Figure 5), assisted rearing (Figure 6) and squares crossed (Figure 7) in a dose dependent manner which indicates its anxiolytic effect by reducing such fearful behavior of animals in open field. Reduction of such fearful behavior of animals in open field test indicates anxiolytic potential of sesamol²¹.

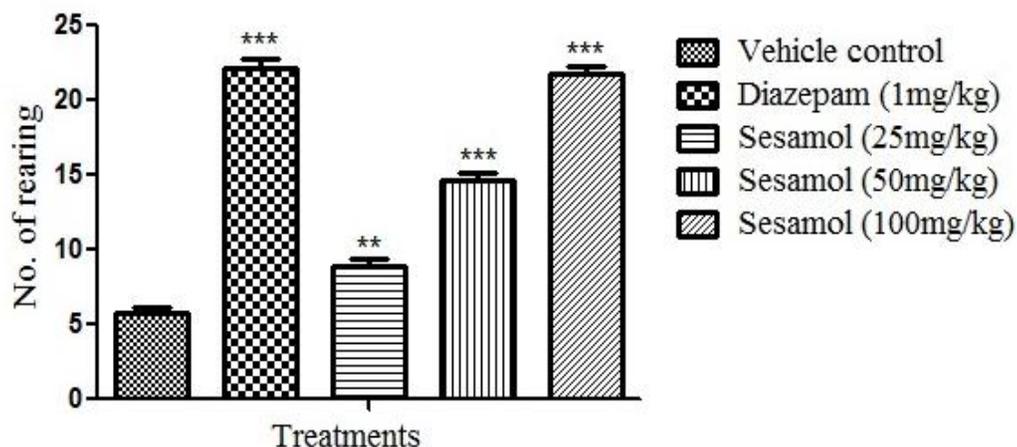


Figure 5: Effect of sesamol on the no of rearing in open field test over a 5 min period of time. All the values are expressed as mean \pm SEM (n = 6); *P < 0.05; **P < 0.01; ***P < 0.001, compared with vehicle control.

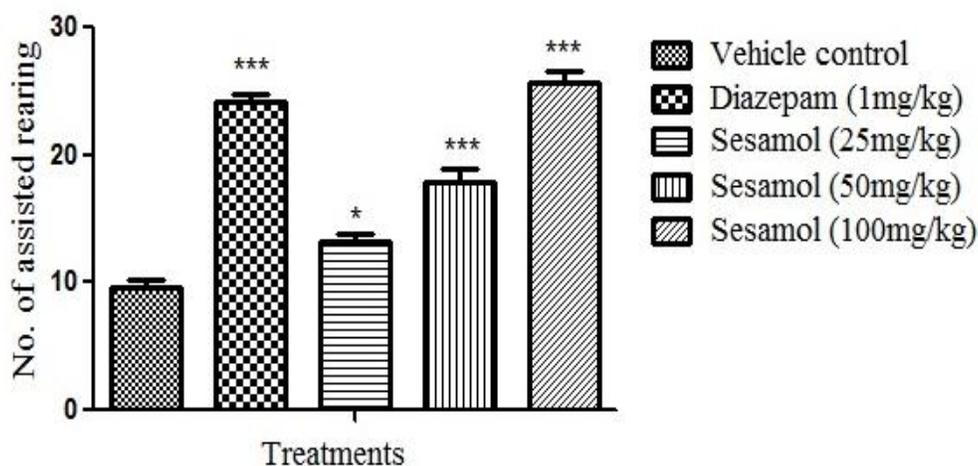


Figure 6: Effect of sesamol on the no of assisted rearing in open field test over a 5 min period of time. All the values are expressed as mean \pm SEM (n = 6); *P < 0.05; **P < 0.01; ***P < 0.001, compared with vehicle control.

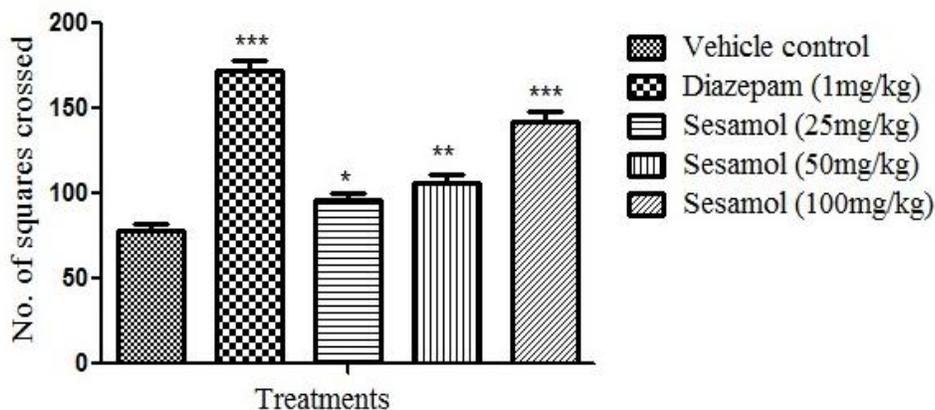


Figure 7: Effect of sesamol on the no of squares traversed in open field test over a 5 min period of time. All the values are expressed as mean \pm SEM (n = 6); *P< 0.05;**P< 0.01;***P< 0.001, compared with vehicle control.

Hole board test:

The results of the hole board test are shown in Figure 8. As shown in Figure, head-dipping behavior was significantly increased by treatment with sesamol (50 and 100 mg/kg) and diazepam (P<0.01, P<0.01 and P<0.05 vs. vehicle, respectively). In hole board test, expression of an anxiolytic-like state may be reflected by an increase in head-dipping behaviors^{22, 23}. Our results showed that sesamol increased the number of head dips significantly indicating an anxiolytic-like effect (Figure 8).

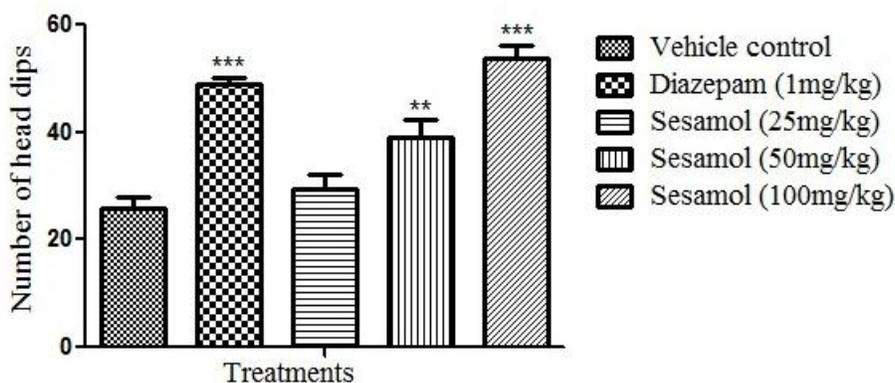


Figure 8: Effect of sesamol on the no of head dipping in hole board test over a 5 min period of time. All the values are expressed as mean \pm SEM (n = 6); *P< 0.05;**P< 0.01;***P< 0.001, compared with vehicle control.

CONCLUSION:

Finally, the combined results of this study allow us to conclude that sesamol exhibited significant anxiolytic activity in elevated plus maze, light and dark model, open field test and hole board test induced anxiety in mice. Further in depth study is needed to understand the mechanism of action

of sesamol at biochemical and physiological level.

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