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ORIGINAL ARTICLE

Screening of Anxiolytic and Antidepressant of Methanolic Leaves Extract of Syzygium Cumini in Mice

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1, 2 Conception & study design
1, 5 Data Collection & Processing
1, 3, 4 Data Analysis and/or interpretation
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ABSTRACT

Background: Anxiety and depression are very common in clinical practice and reduce the overall quality of life. In recent years, various researchers have focused on natural products which are derived from medicinal plants. Studies suggested that diet rich in flavonoids, vitamins and antioxidants are the important components in reduction of anxiety and depression.

Objective: Hence current investigation was aimed to assess the anxiolytic and antidepressant effects of *Syzygium cumini* in mice at 125, 250 and 500 mg/kg.

***Methods*:** These effects were mainly evaluated twice at 8th and 15th days by elevated plus maze, open field test, forced swimming test and tail suspension test.

Results and Conclusion: In open field test *S. cumini* showed escalation rearing in numbers and its duration which indicates improved exploratory behavior and locomotor activity of the animals. In EPM, there was increase in entries numbers and time spent in open arm. Decrease in immobility duration observed at low dose while high dose increased immobility duration in FST. Hence outcomes of current study indicate that *S. cumini* have anxiolytic and anti-depressant effect.

Keywords: Antidepressant, Anxiolytic, *Syzygium cumini.*

INTRODUCTION

Disorders like anxiety and depression are the most common mental illness in health problems. According to WHO, around 450 million people with 12.3% of disease burden globally live with psychological or mental disorders and only a small fraction of these people receive the basic treatment. It is expected that this burden will rise till 15% by 2020 [1]. It has been reported by Anxiety and Depression Association of America (ADAA), 7 out of 10 U.S adults declare that they experience anxiety or stress at average level on daily basis and it's a common part of their life [2]. Currently approximate 340 million people are living with depression worldwide and affecting 21% of world's population [3]. WHO also indicates that unipolar depression will be the 2nd most leading reason of illness induced disability at the end of 2020 [4].

Anxiety and depression can be treated using various therapies. Benzodiazepines are the drug of choice for reduction of anxiety while monoamine oxidase inhibitors (MOIs) and amine reuptake are largely used in the treatment of depression. However, clinically antidepressants are more profound and replace the benzodiazepines due to their role in the treatment of

depression and anxiety [5]. According to previous literature, around two-third of the anxiety and depression patients responded to the available treatment [6]. However there is still need for improvement since, due to complex mechanism of depression, many antidepressants have reduced response and produces severe adverse effects like constipation, dry mouth and sexual dysfunction [7]. Hence there is need of more effective and well tolerated treatment.

Literature suggested that alternative form of medicine for the treatment of psychological ailments has played a major role and the most persistent form of such alternative treatment is herbal treatment. Thus researchers are involved in evaluating effective new molecules of plant origin in different animal models [8-10]. More over in the last 2 centuries, scientific approach for psychoactive plants has been improved particularly after the isolation of psychotropic components.

S. cumini belongs to family *Myrtaceae* with high flavonoids representing the major fraction of phenolic compounds while essential oil of this plant possessed limonene, dipentene and sesquiterpenes of different types as major components [11]. There are several traditional uses of *S. cumini* such as asthma, ulcers, bronchitis, dysentery, biliousness; diabetes and management of its complications [12]. Experimental studies have shown that *S. cumini* leaves extract possess anti-nociceptive activity, anti-inflammatory activity, antioxidant effect, antimicrobial potential, antidiabetic and antihyperlipidemic potential [11, 13].

Literature survey reveals that there are no reports on neurological and behavioral activity of methanolic extract of *S. cumini* leaves was reported. Hence current research was planned to examine the neuro-behavioral potential such as anxiolytic and antidepressant activity of *S. cumini* methanolic leaves extract (SCME) at three different doses in the experimental model of mice.

MATERIALS AND METHODS

Experimental Animals

Swiss albino mice were used to conduct this study with average weight of 25 ± 5g. Animals were housed in suitable plastic cages under precondition temperature of 25 ± 2 °C and were provided easy access to standard diet. The use and care of these animals in our study was in accordance to the guideline presented by National Institute of Health (NIH) [14]. The study was approved by Board of advance studies and researches (BASR) from University of Karachi with reference no BASR/No./02897/Phar

Methanolic extract

S. cumini leaves were collected freshly and proceed to department of botany, University of Karachi for identification with G.H. No. 94236. The leaves were dried for two weeks, after drying; leaves were transformed into powder and macerated with methanol for 14 days. Filtration of mixture was performed which further proceed to concentrate the filtrate using rotary evaporator at reduced pressure [11].

Drug treatment

Animals were distributed in to five groups each group having 10 mice. One group received vehicle and was considered as control group and two groups received SCME in the doses of 150 mg/kg and 500mg/kg. The remaining two groups received imipramine and diazepam 25 mg/kg and 3 mg/kg respectively. Vehicle, SCME and standard drugs were given via oral route for 15 days.

Open Field test

Anxiety and locomotor activity of animal can be assessed using open field test [15]. Individually each mouse was placed in the center of open field. Time for exploration given for each mouse was 5 minutes and ethanol was used to for floor cleaning after each process. Variables estimated were (1) Number of rearing i.e. How many times mouse stood in the air or against the wall with the forelegs, (2) Rearing duration i.e. time duration of mouse in standing situation, (3) Center entries count i.e. when mouse moved center square, (4) Total distance traveled i.e. total number of squares moved by animal multiplied with size of each square. All tests were recorded using Yashica Digital Zoom Camera by placing it above the open field. Oral route was used for daily administration of SCME; vehicle and diazepam for 15 days. Open field test (OFT) was repeated on 8th and 15th day of the study, after 1 hour of giving drugs.

Elevated Plus Maze

Anxiety in animals can be measured by EPM [16]. Each mouse was placed in the EPM center. Five minutes were given to each mouse for exploration and then placed in their cages. Number of entries and

time spent in open and close arms were measured in this test. All tests were recorded by Yashica Digital Zoom Camera from the top view site of the EPM. Oral route was used for daily administration of SCME, vehicle and diazepam for 15 days. This test was done on 8th and 15th day of study, after 1 hour of giving drugs.

Forced Swimming Test

Depression like activity in rodents can be evaluated using Forced swimming test [17]. Cylinder of apparatus was filled with fresh water up to 20 cm height. Temperature of water should be maintained around 25°C. Each mouse was placed in cylinder and activity was observed for 5 min. Replacement of water was done after each animal testing. Variables estimated were (1) Immobility duration, (2) Climbing duration and (3) Swimming duration.[28] SCME, vehicle and imipramine were administered daily through oral route over a period of 14 days and then animals were exposed to pre-test session after 14 days of treatment. Once again animals were placed in the same condition for 5 min (test session) after 24 h of the pre-test session. Test solutions were administered trice a day orally i.e. after the pre-test session, 5 h and 1 h before the main test. All tests were recorded by Zoom Camera (Yashica Digital: EZ F10).

Tail Suspension Test (TST)

A simple, expeditious and reliable method to evaluate the antidepressant potential of drug is tail suspension method. Sherry et al. discussed the method in which immobility duration was induced by tail suspension which is an indication of a depressive state [18]. Each mouse was suspended individually 25 cm from the bottom by adhesive tape applied on the tip of tail. The immobility duration was recorded during the 6 min period and considered immobile when animals hung completely and passively motionless. Oral route was used for daily administration of SCME; vehicle and imipramine for 15 days. This test was done on 8th and 15th day of study, after 1 hour of giving drugs. All tests were recorded by Zoom Camera (Yashica Digital: EZ F10).

Statistical Analysis

Data were demonstrated as mean ± SD and analysis was performed using statistical one-way ANOVA with post hoc test on SPSS version 23. P-values less than 0.05 for significant and 0.005 were considered for highly significant results.

RESULT

Table **1** presents the *S. cumini* extract effect on mice behavior in open field test. Rearing numbers and its duration significantly increased on 15th day at 125 and 250 mg/kg of SC, while highly significant increased at 500 mg/kg on 15th day compared to standard. Duration of rearing was also increased significantly on 8th day and highly significant at 500 mg/kg in comparison with control. Significant increase was observed in distance travelled and no. of center entries on 8th day while highly significant increase in center entries on 15th day at 500 mg/kg dose.

Table 1. Effect of *S. cumini* extract on behavior of mice in Open Field Test.

Groups	Days	Variables			
		No. of rearing	Rearing Duration	Distance travelled (cm)	No. of center entries
Control	8	11.8 ± 1.15	19.5 ± 1.91	2178.0 ± 40.36	6.8 ± 0.58
	15	11.4 ± 0.92	18.8 ± 1.53	2157.0 ± 58.94	7.6 ± 0.51
SC 125	8	14.4 ± 1.50	21.2 ± 2.13	2304.0 ± 97.25	8.6 ± 0.23
	15	18.4 ± 0.81*	28.5 ± 1.34*	2313.8 ± 98.42	9.6 ± 0.51
SC 250	8	15.2 ± 1.12	24.3 ± 1.92	2350.2 ± 47.11	8.9 ± 0.13
	15	20.4 ± 0.91*	30.4 ± 1.51*	2432.0 ± 51.91	10.6 ± 0.65
SC 500	8	17.1 ± 1.35*	26.8 ± 0.86*	2655.0 ± 58.28*	9.4 ± 0.51*
	15	22.6 ± 0.74**	35.2 ± 0.66**	2640.0 ± 39.96*	12.5 ± 0.58**
Diazepam	8	20.4 ± 1.28**	31.2 ± 4.55*	2662.0 ± 162.23*	10.1 ± 0.92*
	15	22.3 ± 1.56**	33.1 ± 2.58**	2666.4 ± 89.25*	14.9 ± 1.07**

n=10, Mean ± S.D

*Level of significance: ≤ 0.05, when compared with control

**High level of significance: ≤ 0.005, when compared with control

Table **2** presents the effect of SCME on mice behavior in EPM. Significant increase in no. of entries in open arm at 250 mg/kg while highly significant increases in no. of entries in open arm at 500 mg/kg were observed on both days. Significant increase in open arm time spent and decrease in close arm observed at 125 mg/kg and 250 mg/kg on 8th and 15th days. However, highly significant increase in time spent in open arm was observed at 500 mg/kg on 8th and 15th days which are comparable to the standard group.

Figure **1** shows the SCME effect in percent comparison from control in mice behavior in FST. Significant decrease and increase in duration of immobility and swimming was observed at 125 mg/kg respectively while significant decrease in climbing duration was observed at 500 mg/kg in comparison with control. Figure **2** presents the effect of SCME on mice behavior in tail suspension test. SCME at doses of 250 and 500 mg/kg showed significant reduction in immobility time on both 8th and 15th days.

Table 2. Effect of *S. cumini* extract on behavior of mice in Elevated Plus Maze.

Groups	Days	Variables			
		No. of entries in open arm	No. of entries in close arm	Time spend in open arm (Sec)	Time spend in close arm (Sec)
Control	8	8.2 ± 0.58	6.6 ± 0.92	131.2 ± 3.59	168.8 ± 3.59
	15	8.4 ± 0.51	5.6 ± 0.51	130.0 ± 4.32	170.0 ± 4.32
SC 125	8	9.8 ± 0.37	6.8 ± 0.44	159.0 ± 4.78*	141.0 ± 4.78*
	15	9.8 ± 0.66	5.9 ± 0.58	155.2 ± 3.58*	144.8 ± 3.58*
SC 250	8	10.9 ± 0.87*	6.1 ± 0.33	168.8 ± 4.32*	131.2 ± 6.71*
	15	11.8 ± 0.67*	5.4 ± 0.43	166.5 ± 3.88*	133.5 ± 5.11*
SC 500	8	13.4 ± 0.67**	5.9 ± 0.58	183.8 ± 5.12**	116.2 ± 5.12**
	15	13.8 ± 0.58**	3.9 ± 0.31*	189.2 ± 4.88**	110.8 ± 4.88**
Diazepam	8	15.6 ± 0.67**	3.4 ± 0.44*	190.6 ± 3.41**	109.4 ± 3.41**
	15	13.2 ± 0.81*	4.2 ± 0.58*	181.1 ± 8.35**	118.9 ± 8.35**

n=10, Mean ± S.D

*Level of significance: ≤ 0.05, when compared with control

**High level of significance: ≤ 0.005, when compared with control

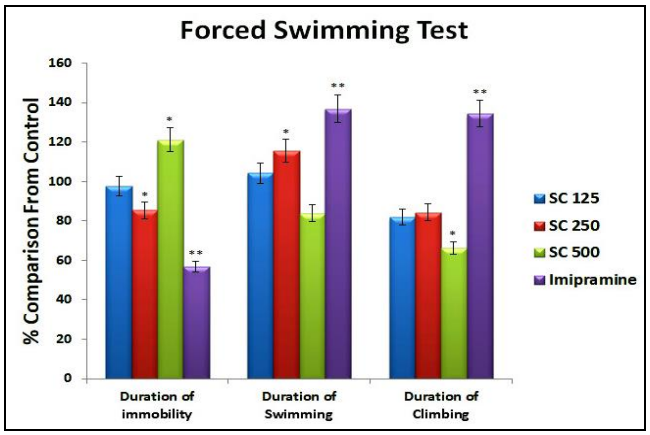

Figure 1. Effect of *S. cumini* extract in percent comparison from control in force swimming test. Data is represented as average values ± SEM, where n=10; *$p \leq 0.05$ significant as compared to control,*$p \leq 0.005$ highly significant as compared to control.

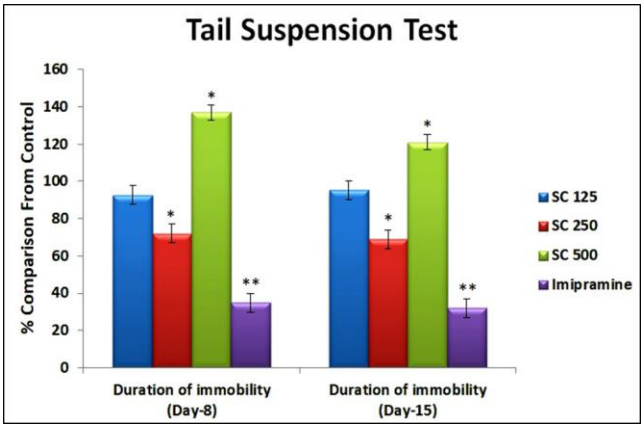

Figure 2. Effect of *S. cumini* extract in percent comparison from control in tail suspension test. Data is represented as average values ± SEM, where n=10; *$p \leq 0.05$ significant as compared to control, *$p \leq 0.005$ highly significant as compared to control.

DISCUSSION

The current study was conducted to assess the anxiolytic and antidepressant effect of SCME at different doses (125, 250 and 500 mg/kg) using EPM and OFT for anxiolytic activity while FST and TST for antidepressant activity.

Highly significant increase was observed at dose of 500 mg/kg in rearing numbers and its duration; these results were almost similar to the standard treatment group which suggests improved locomotor action [19]. While increase in distance travelled at 500 mg/kg on 15th day shows improved motor activity and central nervous system stimulation [20]. These results reflect low anxiety potential and high exploratory behavior of *S. cumini.* In OFT data of percent comparison from control, distance travelled at 500 mg/kg on both days which was similar to the standard treatment while rearing and duration of rearing were greater than the standard group on 15th day which shows the highest locomotor activity at high dose of SCME used in this study.

In EPM 125 and 250 mg/kg showed elevation in open arm time spend however highly significant increase in entries of open arm and time spend at 500 mg/kg on both days in comparison with standard diazepam groups. In EPM data of percent comparison from control, entries in number and time spend in open arm at 500 mg/kg were more than standard group on 15th day while entries in number and time spend in close arm at 500 mg/kg were reduced on 15th day.

All these behavioral actions of SCME can be perceived with respect to CNS stimulant and anxiolytic effects. Hence such behavior is the actual interpretation of increased locomotory response by treated animals [21]. Due to the similar type of phenotype, it is very difficult to distinguish the underlying mechanism of CNS stimulant and anxiolytic drugs. It also has been reported that various plant species possessed anxiolytic activity due to the presence of flavonoids, used in folk medicine [22]. Therefore, anxiolytic effects of SCME could be linked with their flavonoids content which modulates neurological processes involved in anxiety particularly Gamma-Amino Butyric Acid (GABA) receptors [23].

Depression can be distinguished by neuroendocrine, psychosomatic and somatic symptoms. Most commonly method used for evaluation of depression in animals are FST and TST. Thus some phases of human depression resemble with the immobility behavior of animals during these tests [24]. Immobility is one of the variables in FST and TST that indicate hopeless and depressive behavior of the animal. Reduction in immobility period shows anti-depressant potential [25]. When compared with control, 125 and 250 mg/kg have shown reduction in duration of immobility however, these doses increase the duration of swimming which in turn counter balance the immobility duration. This behavior was also observed in case of imipramine group and it has also been reported that imipramine effects are associated with decrease in duration of immobility which is further compensated by improved swimming duration [26].

Figure (**3** and **4**) showed reduction in immobility duration in a dose dependent manner at 125 and 250 mg/kg however duration of immobility was increased at a dose of 500 mg/kg which indicates its depressive effect and it could be due to the presence saponins in high levels in the SCME [27]. Role of saponins was reported in various studies as CNS depressant due to its agonistic response on GABA-A receptor complex [28, 29]. Hence therapeutic levels of drug concentration can be achieved by the adjustment of dose. Although this study design does not explain about the active moiety through which pharmacological effects are possible but on other side, it let us imitate experimentally a condition analogous to the addition of the *S. cumini* in the human diet.

Composition of flavonoids in SCME leaf is approximately 451.50 ± 9.85 mg/g, while 23–68.2 mg/day is the average intake of flavonoids in adult [12, 30]. Therefore, in this study both doses of *S. cumini* leaf extract were used to accomplish the daily needs of flavonoids. Indeed, flavonoids are, in part, synergistic concerning its biological actions.

CONCLUSION

Our results make evident that the leaves of SCME possess good anxiolytic on all three doses while antidepressant properties on two doses. As per obtained results of this study allows us to propose this plant to choice for isolating new active moieties with potential antidepressant and anxiolytic activity. Although further clinical investigations are necessary but these results are promising since *S. cumini* might be considered as an alternative for the treatment of

such mental illness to other medications currently used.

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