

ASSESSMENT OF SEDATIVE ACTIVITY OF SYZYGIUM CUMINI LEAVES ON ANIMAL MODELS – AN IV-VIVO DESIGN

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ABSTRACT

In a biological system, a sedative is defined as any substance that induces calmness or sleep is called sedative. Recently, increasing attention has been focused on the use of natural sedatives. The aim of present study is to evaluate the sedative activity of *Syzygium cumini* leaf extracts on animal experimental models using rota rod apparatus and Hole cross test. In the recent years, the use of herbal products has been increasing in developing countries. *Syzygium cumini* is one of the widely used medicinal plants in the treatment of various diseases in particular diabetes. The present review has been primed to describe the sedative effect of *Syzygium cumini* leaf extracts, traditional uses,

chemical constituents and pharmacological actions. The evaluation of the sedative activity was performed in the Rota rod apparatus and Hole cross test. The leaves are claimed to contain glycosides, flavonoids, phenolic acids, triterpenoids, myricetin, quercetin, tannins etc. The vast number of literatures found in the database revealed that the extracts of different parts of Jambolan showed significant pharmacological actions. We suggest that there is a need for further investigation to on the sedative effect of Jambolan leaf extracts, which confer the pharmacological action. Hence evaluation of sedative activity is useful for producing drugs in the treatment of various diseases.

KEYWORDS: *Syzygium cumini*, sedation, Swiss Albino mice, Rota rod apparatus, Hole board test.

INTRODUCTION

Sedative and hypnotics are the drugs which can reduce anxiety and produce a calming effect by inducing the onset of sleep as well as maintaining sleeping duration.^[1,2] Nowadays, these drugs are extensively used in treatment of different psychiatric disorders including anxiety and insomnia. However, continuous use of these currently available sedative-hypnotic therapies tends to have serious side effects ranging from respiratory, digestive, and immune system dysfunctions to deterioration of cognitive function, physical dependence, and tolerance.^[3]

Thus, development of new sedative-hypnotic drugs with fewer side effects has been suggested to be a promising approach to combat different psychiatric disorders. The *Syzygium cumini* is one of the genera of the myrtle family Myrtaceae, which is native to the tropics, particularly to tropical America and Australia. It has a worldwide, although highly uneven, distribution in tropical and subtropical regions. The genus comprises about 1100 species, and has a native range that extends from Africa and Madagascar through southern Asia east through the Pacific. Its highest levels of diversity occur from Malaysia to north-eastern Australia, where many species are very poorly known and many more have not been described taxonomically. Plants of this family are known to be rich in volatile oils which are reported for their uses in medicine^[4] and many fruits of the family have a rich history of uses both as edibles and as traditional medicines in divergent ethnobotanical practices throughout the tropical and subtropical world.^[5]

Some of the edible species of *Syzygium* are planted throughout the tropics worldwide. The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparations have been found to be effective in the treatment of diseases. Therefore to meet the increasing demand of manufacturing modern medicines and export, the need of the medicinal plants have enormously increased. This demand is generally met with by cultivating uprooted medicinal plants.^[6] Based on traditional uses, infusions or decoctions of leaves of Jambolan in water at an average concentration of 2.5 g/L and drank it in place of water at a mean daily intake of about 1 litre are used in the treatment of diabetes.^[7] The juice of leaves is given orally as anti-dote in opium poisoning and in centipede bite.^[8] The leaf extract is also used to treat renal problems.^[9] The leaves are also used to strengthen the teeth and gums, to treat leucorrhoea, stomachalgia, fever, gastropathy, strangury, dermopathy^[10], constipation and to inhibit blood discharges in the faeces.^[11]

To understand sedative/hypnotics has been to hypothesize that sleep results from drug-induced reduction in energy metabolism.^[12] Sedative medications demonstrate the alternating ultradian rhythm of NREM and REM sleep, which indicates an active regulatory mechanism. It seems more parsimonious, then, to hypothesize that sedative/hypnotics act at specific sites involved in sleep regulation, rather than producing a nonspecific “slowing” of the nervous system.^[13]

Balanced sedation can result increased co-operation, and patients are more likely to mobilize and wean from mechanical ventilation earlier than over-sedated patients.^[14] One of the most important goals of clinicians is patient comfort. When patients present to the emergency department (ED), treating the pain and anxiety that accompany the chief complaint are critical to patient satisfaction and quality of care. Nonetheless, clinicians may underuse sedation, usually from a lack of experience or from unchallenged myths regarding its use. Sedation is the depression of a patient's awareness to the environment and reduction of his or her responsiveness to external stimulation.

Preliminary phytochemical screening revealed that this plant is rich in flavonoids and terpenes including (4-O-acetyl)-alpha-L-rhamnopyranosides of mearnsetin (myricetin-4-methyl ether) and myricetin 3-O-(4-acetyl-2-O-galloyl)-alpha-L-rhamnopyranoside from leaves extract. Additionally identified terpenoids of broom weed involve alpha-amyrin, friedelin, glutinol, and ifflaionic acid, where all of these phytochemicals were reported to show their biological activity against different pathological conditions.^[15-18] However, so far, there is no report demonstrating the sedative-hypnotic activity of *Syzygium cumini*, which prompted us to design the present study to evaluate the role of this plant on the central nervous system (CNS) in mice. We found that the leaf extract of *Syzygium cumini* significantly reduced the locomotor activity and motor coordination of mice. Therefore, our findings strongly support the sedative activity and suggest that it can be useful to treat different psychiatric disorders including insomnia.

The present aim of the research work is to evaluate the sedative activity of *Syzygium cumini* leaf extract by using various solvents.

MATERIALS AND METHODS

Collection of plant material

The plant (*Syzygium cumini*) leaves required for the study was collected from our college. The collection of the leaves was carried out a month earlier.

Preparation of plant extract

The collected fresh plant leaves were washed thoroughly with water and then air dried for a week at 35-40 °C and pulverized in electric grinder. The grinded powder is sieved with a 40# Sieve. The powder was then extracted in Soxhlet apparatus using ethanol for 24 hours. Then the solution was filtered and the extract was concentrated and dried by means of rotary evaporation. The marc obtained is dried and used in the extraction procedure for other solvents.^[19-21]

Animals

For the experiment Swiss albino mice of either sex, 4-5 weeks of age, weighing between 25-30gm, were collected. Animals were maintained under standard environmental conditions (temperature: 24.0 ± 1.0 °C), relative humidity: 55-65% and 12 hrs light/12 hrs dark cycle) and had free access to feed and water. The animals were acclimatized to laboratory condition for two weeks prior to experimentation.^[22] The experiment protocol was approved by an Institutional Animal Ethics Committee of School of Pharmacy, Anurag Group Institutions and care of the animals was taken as per guidance of the Committee for the Process of Control and Supervision of Experiments on Animals (Reg. no: I/IAEC/AGI/020/2018).

Drugs and treatment

After reconstituted in distilled water all the extracts were administered to the mice at 100 and 200 mg/kg per orally by gavage. The water (5 ml/kg) was administered by gavage to the control group. All drugs, used as standard, were dissolved in 0.9% saline and administered intraperitoneally (i.p.). Diazepam (3 mg/kg i.p.) was used as standard CNS depressant drug.

Soxhlet extraction

The *Syzygium cumini* leaves were collected and dried in shade and made into powder. About 75gm of powder was weighed and kept in muslin cloth and placed in thimble. To this 50ml of ethanol is added and 250ml ethanol is taken in round bottom flask and temperature is set for 78°C. The cycles are continued till the product colour disappears and extraction is completed.

The extract is then collected and concentrated by rota evaporator.^[23] The obtained residue is tested for phytochemical constituents and for presence of sedative activity.



Figure 1: Soxhlet extraction.

Table 1: Grouping of animals.

Groups	Dose/route of administration
Group -1 (controlled)	Distilled water
Group-2 (standard)	Diazepam 3mg/kg (i.p.)
Group-3 (leaf aqueous extract)	100mg/kg (p.o)
	200mg/kg (p.o)
Group-4 (leaf chloroform extract)	100mg/kg (p.o)
	200mg/kg (p.o)
Group-5 (leaf ethanol extract)	100mg/kg (p.o)
	200mg/kg (p.o)
Group-6 (leaf ethanol extract)	100mg/kg (p.o)
	2000mg/kg (p.o)

Pharmacological screening

Rota rod apparatus

The Rota rod used in this test was a metallic rod (3 cm diameter) coated with rubber and connected to a motor. The rod was rotated at a constant speed i.e. 9 r/min and was about 60 cm above the tabletop in order to prevent the mice from jumping off the roller. Mice were exposed to Rota rod as a protest before the experiment and only those mice that remained on the rod for 2 min at a speed of 9 rpm were included in the study. All the groups ($n = 6$) were treated (i.p.) with diazepam (3mg/kg), distilled water (2 mL/kg), and various solvent fractions

at the dose of 100 and 200 mg/kg, i.p. 30 min before the experiment. Each mouse was allowed for 2 min on the revolving rod and the time spent on the rod was recorded.^[24,25]

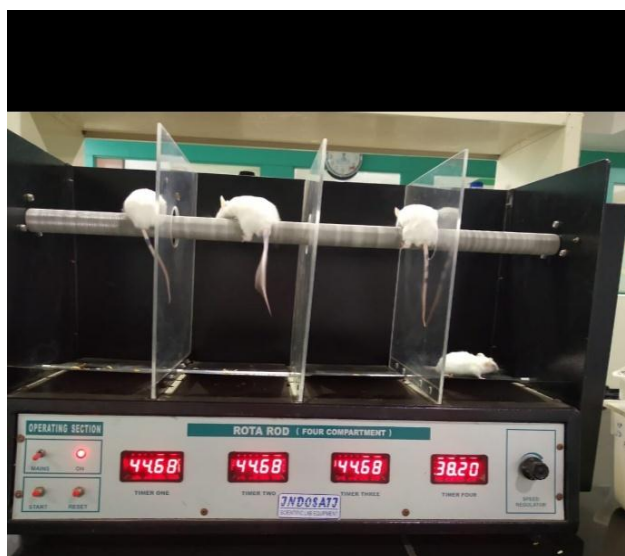


Figure 2: Rota rod testing.

Hole board test

The hole-board test was performed according to the previously described^[26], with slight modifications. For this test, we used a flat platform of 60 cm × 30 cm in diameter with 16 evenly spaced holes. In brief, after Diazepam administration and vehicle administration, each animal was allowed to move on the platform and the number of head dips into the holes was counted for 5 min.



Figure 3: Hole board test.

Statistical analysis

All values are expressed as Mean±SEM for all the models. Data was analysed by one way ANOVA followed by Newman-Keuls test. The result was considered to be statistically significant when $p < 0.05$.

RESULTS

Table 2: Rota rod test results.

		Aqueous extract	Chloroform extract	Ethanol extract	Ethyl acetate extract
Low dose without drug	Mean	1.850	2.038	1.850	1.950
	Standard deviation	±0.327	±0.051	±0.327	±0.242
Low dose with drug	Mean	1.333	1.465	1.333	1.450
	Standard deviation	±0.344	±0.287	±0.344	±0.288
High dose without drug	Mean	2.050	2.050	2.050	0.050
	Standard Deviation	±0.104	±0.083	±0.104	±0.104
High dose with drug	Mean	1.135	0.850	0.531	1.268
	Standard Deviation	±0.161	±2.273	±0.237	±0.299

Each value is presented as the mean \pm SEM, various extract of *Syzygium cumini* leaves.

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs control group (Newman-Keuls test).

Effect of Aq extract on muscle strength by using Rotarod

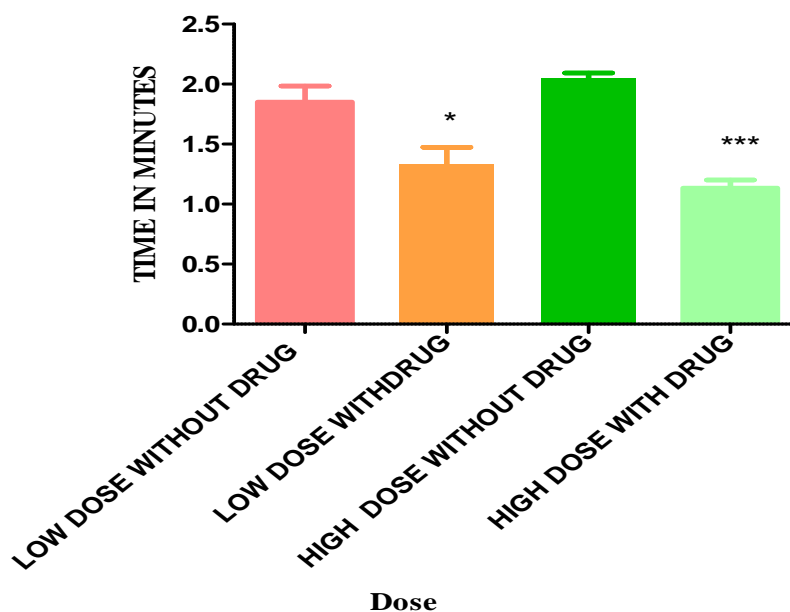


Figure 4: Effect of aqueous extract by using Rota rod.

Effect of Ethanol extract on muscle strength by using Rotarod

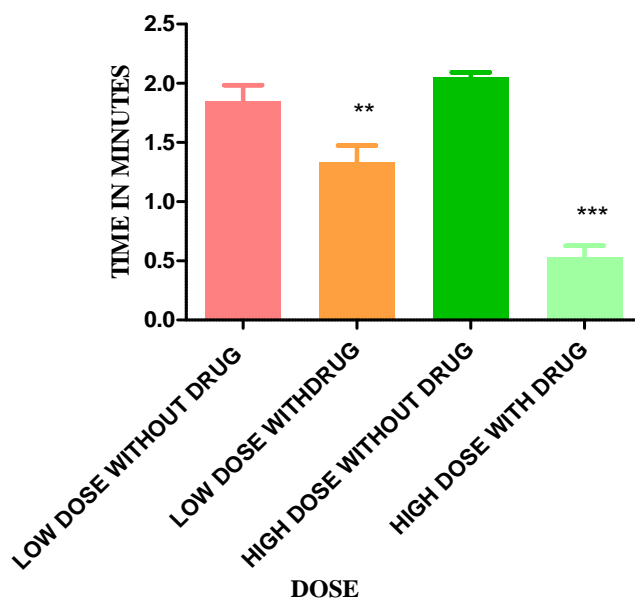


Figure 5: Effect of ethanol extract by using Rota rod.

Effect of chloroform extract on muscle strength by using Rotarod

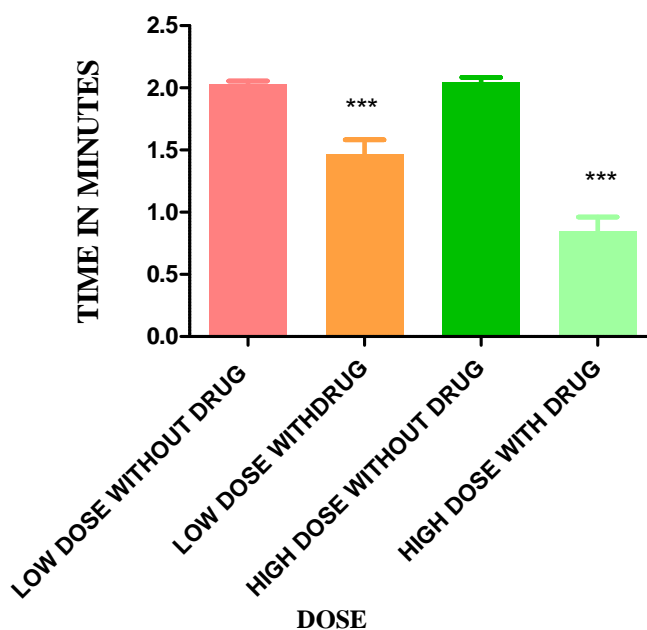


Figure 5: Effect of chloroform extract by using Rota rod.

Effect of Ethyl acetate extract on muscle strength by using Rotarod

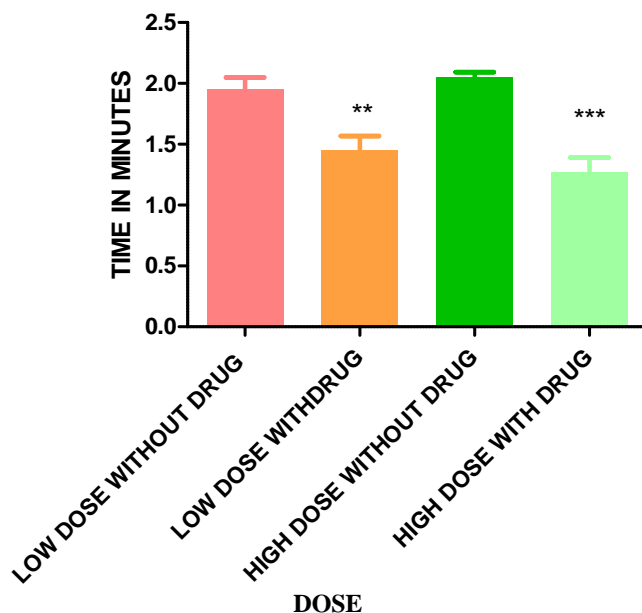


Figure 6: Effect of ethyl acetate extract by using Rota rod.

Table 3: Hole board test results.

		Aqueous extract	Chloroform extract	Ethanol extract	Ethyl acetate extract
Low dose without drug	Mean	24.17	23.00	23.00	23.00
	Standard deviation	±3.312	±1.789	±1.789	±1.789
Low dose with drug	Mean	10.67	11.83	8.000	11.83
	Standard deviation	±0.8165	±1.169	±1.265	±1.472
High dose without drug	Mean	23.17	22.67	22.67	22.67
	Standard Deviation	±2.858	±1.211	±1.211	±1.211
High dose with drug	Mean	5.667	6.000	2.667	8.000
	Standard Deviation	±1.506	±1.095	±0.8165	±1.265

Each value is presented as the mean ± SEM, various extract of Syzygium cumini leaves.

****p < 0.001, **p < 0.01, *p < 0.05 vs control group (Newman-Keuls test).*

Effect of Aq extract on exploratory behaviour of mice

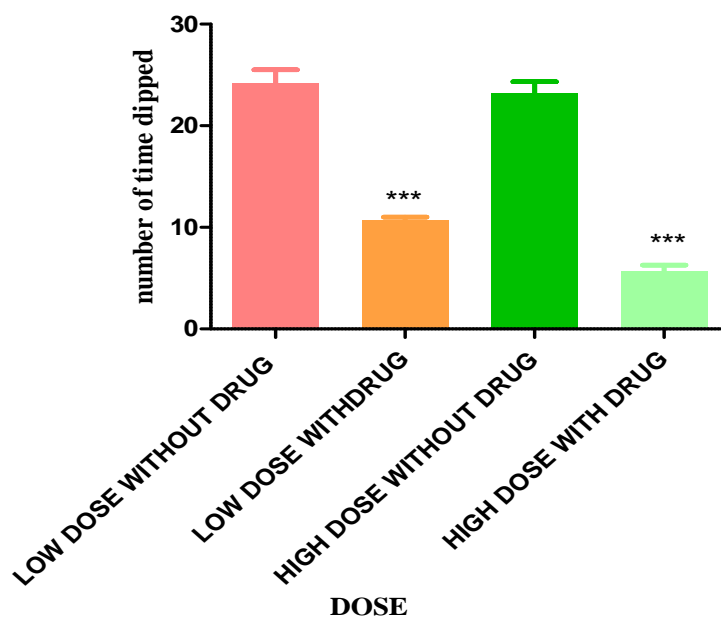


Figure 7: Effect of aqueous extract on exploratory behaviour.

Effect of chloroform extract on exploratory behaviour of mice

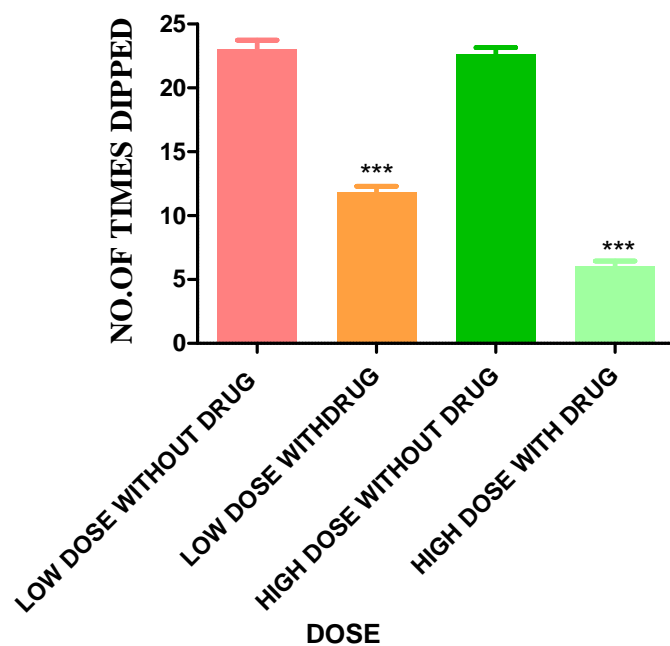


Figure 8: Effect of chloroform extract on exploratory behaviour.

Effect of ethanol extract on exploratory behaviour of mice

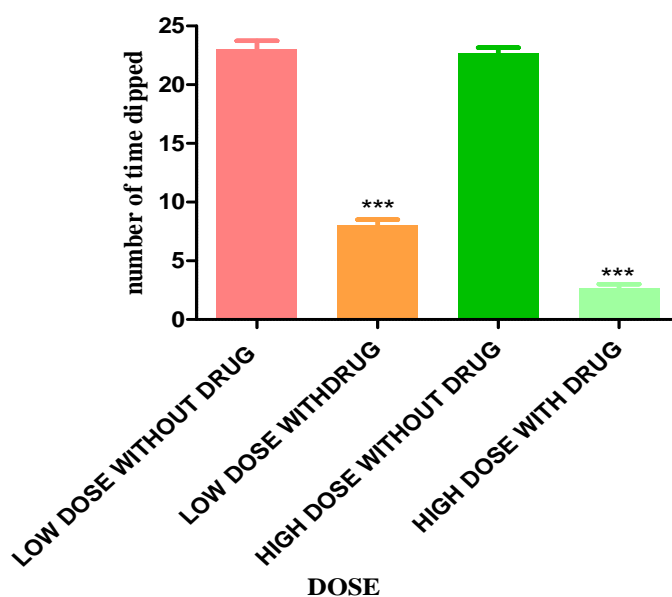


Figure 9: Effect of ethanol extract on exploratory behaviour.

Effect of Ethyl acetate extract on exploratory behaviour of mice

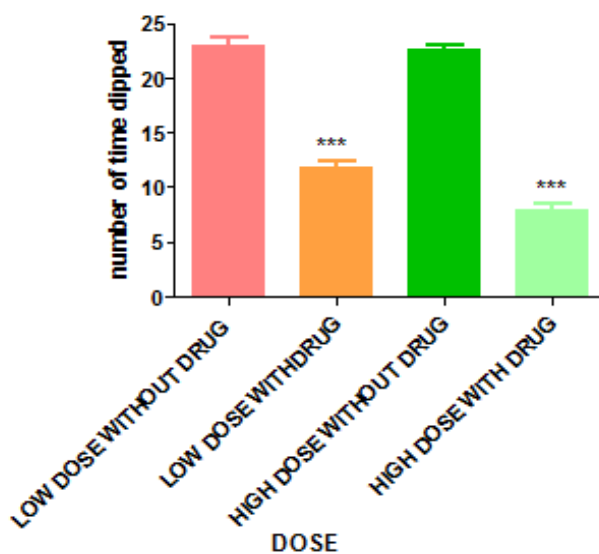


Figure 10: Effect of ethyl acetate extract on exploratory behaviour.

DISCUSSION

Plants due to the presence of many secondary metabolites might show different types of bioactivities. Usually bioactive compounds are toxic in higher doses. So, the lethality originated from toxicity is considered as a marker of bioactive compounds. For this purpose the brine shrimp lethality is utilized as bench top bioassay to monitor the presence of

bioactive metabolites in plant.^[27,28] It also justifies the use of this plant for treating many pathological states as used by the traditional healers.

The current neuropharmacological study with the various extracts of *Syzygium cumini* has been conducted. The plant extract demonstrated central nervous system depressant activity as indicated by the decrease in locomotor activity in mice in Hole cross, Rota rod test. The efficiency of the plant extract was almost similar to that of common sedative drug diazepam. Central Nervous System (CNS) stimulating drugs accelerate motor activities such as locomotion, grooming and rearing behaviour, while the CNS depressing drugs inhibit those actions. Our present study showed the significant level of inhibition of the spontaneous motor activities in mice which ultimately indicates the effective induction of sedation by the studied plant extract through CNS depression previously it was reported that the CNS depressants prolong the drug induced sleeping time. The studied plant-extract increased diazepam induced sleeping time compared to the normal saline treated group. This ability to prolong the sleeping time demonstrates the capability of the *Syzygium cumini* to cause CNS depression.

However, plant extract usually contains numerous biosynthetic compounds. These might show additive or synergistic action on single or multiple target sites for displaying various types of bioactivities. Many plant extracts have already been reported to act as ligands for GABA-A receptor for showing the sedative and hypnotic actions. From the *Syzygium cumini* some triterpenes and steroids have been isolated, which might be responsible for acting as ligands for GABA-A receptor and displaying sedative action.

CONCLUSION

In conclusion, the present findings in our study indicate that all the tested doses of different extracts of *Syzygium cumini* have exhibited sedative effect. The effect is dose dependent, long lasting and statistically significant. Sedation principally mediated by the GABA receptor complex in the CNS, which is also involved in other physiological functions related to behaviour, and in several psychological and neurological disorders. Furthermore, evidence obtained from the present study may justify that the use of *S. cumini* in traditional medicine for the treatment of insomnia, depression, and neurodegenerative diseases. However, further studies are needed to isolate the bioactive compound(s) and demonstrate the precise molecular mechanisms responsible for the pharmacological activities.

There is a need for further evaluation of different fractions of various extracts of *Syzygium cumini* leaf as a potential remedy for the treatment of various diseases. This knowledge about the medicinal plants usage can also be extended to other fields like field of pharmacology. This study is among the early research in order to justify potential pharmacological properties of *Syzygium cumini* leaves for its sedative activity. It is hoped that the finding of this research would be beneficial and contribute to the development of better medicines from phytochemicals.

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CONFLICT OF INTEREST

Author declares that there is no conflict of interest to disclose.

SOURCE OF SUPPORT

Nil.

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