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PHARMACOGNOSTIC SCREENING AND HYPNOTIC ACTIVITY OF LEAVES EXTRACT OF *ADENIUM OBESUM*

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ABSTRACT

Insomnia is a problem with the depth or duration of sleep, and difficulties initiating or maintaining sleep are frequent symptoms. The research focuses on the phytochemical and hypnotic screening of leaves extract of *A. obesum* in experimental animal models. The fresh leaves of *A. obesum* were collected from Meerut region and authenticated by the botanist. The leaves were washed to make these dust-free; dried in shade. Ethanol (70%) was used as solvent in extraction. 200ml of ethanol (70%) was used for 50gm quantity of powder. Various phytoconstituents were looked for in the plant extracts. Animal House at the Translam Institute of Pharmaceutical Education and Research in Meerut will supply Wistar rats of either sex weighing between 150 and 200g. There are four sets of animals (n=6) i.e., group 1 administered distilled water; group 2 administered Diazepam (4 mg/kg) orally; group 3 administered *A. obesum* methanolic leaf extract (100mg/kg) orally; group 4 administered *A. obesum* methanolic leaf extract (200mg/kg) orally once daily for 21 days. Various parameters including Thiopental sod. induced sleeping time, Motor behaviour, Light and Dark Arena and Actophotometer were evaluated for hypnotic activity. Based on dried extract, the percentage yield was calculated as 69.26% when weight of practical yield was compared with theoretical yield. Leaves exhibited alkaloid, flavonoids, glycosides, amino acids as chemical constituents. It also exhibited for tannins in some tests. In results, it was found that *A. obesum* methanolic leaves extract is very significant in the preclinical treatment of insomnia. Its mode of action might involve inhibiting neurotransmitter release and encouraging catecholamine breakdown. In conclusion, *Adenium obesum's* methanolic leaf extract is effective in reducing insomnia and resetting the mind to promote sleep. It suggests conducting clinical research to assess the hypnotic effects of *A. obesum*, which would have been utilized to treat insomnia and other associated issues.

KEYWORDS: *Adenium obesum*, hypnotic activity, diazepam, thiopental sodium induced sleeping time, locomotor activity.

INTRODUCTION

Insomnia is a problem with the depth or duration of sleep, and difficulties initiating or maintaining sleep are frequent symptoms. These symptoms can cause severe distress and make it difficult to function during the day (Ohayon and Reynolds, 2009). Sleep disorders like insomnia are linked to poor health outcomes like a lowered quality of life and physical and neurological conditions (Morin et al. 2015). The hypothesized mechanism for relationship b/w improper sleep and diabetes & CVS disease in AI/AN are similar to individuals in other ethnic communities (Ip et al. 2007; Spiegel et al. 2004). It is chronic problem for some people as 74% sufferers experience symptoms for at least 1 year. Female are more prone to develop insomnia. More than 1/2 half of the persons in 3-year study remitted indicating 27% recurrence rate. One-third of the population have been suffering from insomnia in their family (Beaulieu Bonneau et al., 2007), while another third has had insomnia themselves. Women are prone to report insomnia than men and daytime repercussions and diagnosed with insomnia (Kim et al. 2000).

Low socio-economic status, education-deprived, and being single are all associated with insomnia. It's also linked to bodily issues, with half of those who have sleeplessness also having various physical issues. People who suffer from sleeplessness are more likely to be unhappy with their health (Maggi et al. 1998). Insomnia is significantly linked to mental illnesses, the most frequent of which are depression, anxiety and post-traumatic stress disorder. Most patients with serious

depression report insomnia across cultures, and those who suffer sleeplessness are more likely to have a gloomy mood (Foley et al. 1995).

Plant profile

Plants & plant exudates have been steadily rising in traditional systems such as homoeopathy, acupuncture, aromatherapy, and the ayurvedic medicinal system since ancient times (Martins, 2013). Although *Adenium obesum* first obtained in Africa, but now found across the tropics and sub-tropics. The chosen plant species can be found in both Asia and Africa. Oman is one country where many species are found in plenty and therefore Oman is the home to the desert rose. All parts of a given species are used to treat a wide range of illnesses. Several plant species have been singled out for commercial cultivation due to their medicinal value (Akhtar et al., 2017).

Taxonomy

Kingdom: Plantae
Class: Magnoliopsida
Order: Gentianales
Family: Apocynaceae
Genus: *Adenium*
Species**:** *obesum*

Fig. 1: Depiction of *A. obesum* plant.

Stem and bark exhibited the chemical constituents as Betulin and Rosmarinic Acid. Stem showed 3,5,7,3,4,5-Hexahydroxy flavone and 5,7,3,4-Tetrahydroxy flavone whereas leaves confirmed for various chemical constituents i.e., Honghelin, Obeside-B&C (Amin et al. 2013; Hossain et al. 2013) Based on ROL, I found that hypnotic potential of leaves extract of *A. obesum* did not evaluate. This research focuses phytochemical and hypnotic screening of leaves extract of *A. obesum* in experimental animal models.

MATERIALS AND METHODS

Experimental requirements

Leaves of *Adenium obesum*, thiopental sod., Diazepam, distilled water, Wistar rats, and ethanol.

Collection and authentication of plant

The fresh leaves of *A. obesum* were collected from Meerut region and authenticated by the botanist. The leaves were washed to make these dust-free; dried in shade.

Preparation of extract

Ethanol (70%) was used as solvent in extraction. 200ml of ethanol (70%) was used for 50gm quantity of powder (George, et al; 2016). The extract was prepared by following steps-

Leaves of *A. obesum*were collected
↓
Shade dried for 15 days
↓
Crushed using grinder and thus powdered
↓
The powder extracted thru Soxhlet apparatus for 48 hours
↓
Slurry obtained
↓
Dried on water-bath (40ºC)
↓
Dried extract obtained

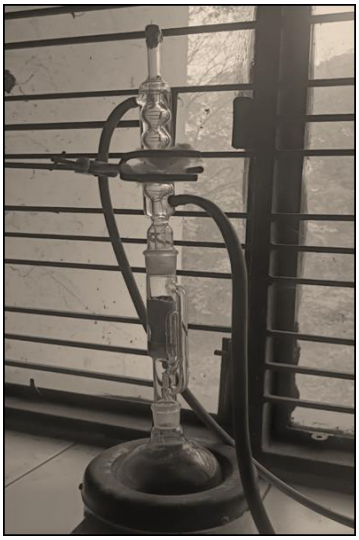

Fig. 2: Soxhlet extraction.

Extracts are kept on water bath at 40ºC to evaporate the solvent content and concentrate the drug. After the drug was poured into petri-dish to obtain the solid drug extract. The obtained mixture was dried under vacuum using water bath. The percentage yield of the extract of the *A. obesum* leaves is calculated thru below mentioned formula (Khan et al. 2020) –

$$\text{Percentage Yield} = \frac{\text{Actual Yield}}{\text{Theoretical Yield}} \times 100\%$$

Phytochemical Screening

Various phytoconstituents were looked for in the plant extracts.

Alkaloids Detection

Filtered solutions of extracts in diluted HCl were prepared separately. As part of the Mayer's test, filtrates were exposed to Potassium Mercuric Iodide (Mayer's reagent). The presence of alkaloids is indicated by the formation of a yellow precipitate.

Wagner's Test: Iodine in potassium iodide (Wagner's reagent) was used to treat filtered samples. When a brown or reddish precipitate forms, alkaloids are present.

The filtrates were given the Hager's Test using Hagers Reagent. When alkaloids are present, ppt turns yellow.

Detection of Glycosides

Fehling's test: With distilled water dilution, Fehling's solutions A and B were heated for one minute. There were 8 drops of plant extract added to this transparent blue solution. It was then combined with 1 ml of Fehling's solution and heated for 5 minutes in a water bath. Brick red precipitation is an indication of glycoside content.

Detection of Saponins

For the foam test, 2 grams of plant extract were added to 10 milliliters of distilled water and the mixture was shaken until a stable, persistent froth formed. Saponins are present when foam forms.

Tannin Detection

For the ferric chloride test, 0.5 grams of the dried powdered sample was reconstituted by boiling it with 20 milliliters of water in a test tube. A few drops of 0.1% FeCl3 were added and the resulting color was either brownish green-black or blue-black.

For the lead acetate analysis, two milliliters of plant extract was mixed with two milliliters of water. This mixture was then added to 0.01g of lead acetate and vigorously shaken. Tanning compounds are present when white turbidity and precipitate form.

Flavonoid Detection

A little sample of extract was treated with aqueous NaOH and HCl, and the resulting golden orange hue was noticed as part of the NaOH test.

When a sample of the extract was treated with concentrated hydrosulfuric acid (H2SO4), the resulting orange color was seen.

Terpenoids Detection

After adding 2.0 ml of chloroform to 5 ml of the aqueous plant extract and evaporating the mixture, on the water path, and boiled with 3 ml of concentrated H2SO4. As terpenoids took shape, a grey colour emerged.

Detection of Steroids
The 5 ml of aqueous plant crude extract was mixed with 2 ml of chloroform and concentrated H2SO4. Red coloration emerged in the base chloroform layer, implying the presence of steroids.

Detection of carbohydrates
Molish's Test
Add a few drops of a -naphthol solution in alcohol to 2-3 ml of extract from each solvent, shake, and then pour concentrated H2SO4 from the sides of the test tube. There's a violet ring where the two liquids meet.

Faltin's assay
It is put to use in the quest for lessening sugars. Solution A consists of 34.66 grams of copper sulfate dissolved in 500 milliliters of distilled water. Solution B is 50 milliliters of water containing 17.3 grams of potassium sodium tartrate and 50 milliliters of pure water. Mix two solutions together before using. A mixture of 1 milliliter of Fehling's A and B solution should be cooked for 1 minute. To this, add an equal volume of the test solution. Cook for 5-10 minutes in a kettle of boiling water. The color changed from yellow to a bright red.

Animal Preparation
Animal House at the Translam Institute of Pharmaceutical Education and Research in Meerut will supply Wistar rats of either sex weighing between 150 and 200g. The animals are kept in ideal conditions, with a 251°C temperature range and a 12-hour light/dark cycle. Standard rat pellet meal and water are provided ad libitum, and the relative humidity is kept between 44-56%. Rats are fasted for one hour before experiments (Bhajoni et al., 2016).

Group design
There are four sets of animals (n=6) with the following compositions:
Group 1 administered distilled water for 21 days.
Group 2 administered Diazepam (4 mg/kg) orally once daily for 21 days.
Group 3 administered *A. obesum* methanolic leaf extract (100mg/kg) orally once daily for 21 days.
In Group 4 administered A. obesum methanolic leaf extract (200mg/kg) orally once daily for 21 days.

Evaluation of hypnotic potential
Thiopental sod. induced sleep
Using a previously reported method, we determined how long it took for thiopental sodium to produce sleep. Each rat received 20 mg/kg of intraperitoneal thiopental sodium (TS) 30 minutes after vehicle or EESD therapy, and 15 minutes after diazepam administration. It was noted that animals experienced a temporary loss of equilibrium. Injection of thiopental sodium produces reflex recovery shortly afterward (latent response period and sleep length; time between loss and return of sleep (Turner, 1965).

Motor behaviour
In this experiment, we employed a horizontal spinning rod (Ugo Basile, Varese, Italy) that made 20 revolutions per minute. Mice that were able to maintain their grip on the rod for more than 180 seconds were selected and categorized accordingly. Half an hour after receiving the vehicle or medication, and 15 minutes before the experiment, each mouse was placed on the rod for 180 seconds. Each mouse's descent time from the top of the spinning pole was then recorded. Time limits of three minutes were established (Fujimori & Cobb, 1965).

Light and Dark Arena
A 100-watt lamp is placed 30 centimeters above the bottom of the box to simulate a light-dark arena. The rats spend 5 minutes exposed in the box's bright center. We keep track of how many people enter the light arena and how long they stay there for up to five minutes. Before housing a new rat, it is always well cleaned (Khan et al., 2020).

Actophotometer
For reliable readings, the actophotometer must first be turned on and checked to ensure that all photocells are operational. The rats spend 10 minutes in the activity cage once per experiment. Each rat's activity level is monitored for the first 10 minutes. When all else fails, a comparison between Imipramine, a typical medication, and the patient's motor activity might be made (Kulkarni, 1999).

RESULTS AND DISCUSSION

Percentage yield
Based on dried extract, the percentage yield was calculated as 69.26% when weight of practical yield was compared with theoretical yield.

Phytochemical constituents
The following table shows the list of chemical constituents that were observed in the leaves extract of *Adenium obesum*-
Foam test for foam test was found positive that indicated for its saponins. Moreover, all the tests for alkaloids i.e., Mayer's test etc. were found positive when observed. Tests for carbohydrates i.e., Fehling's test and Benedict's test were found positive that proved for its carbohydrates content. *Adenium obesum*showed absence of tannins when examined through its tests. Tests for proteins i.e., Biuret test exhibited the presence of proteins. The positive sign of Keller-killiani test confirmed for its glycoside content.

Terpenes were found absent; however, flavonoids were found presence when observed in lead acetate test.

Table 1: Phytochemicals of *Adenium obesum*extract.

Phytochemicals	Test	Ethanolic extract of *A. obesum*
Saponins		
	Foam test	+
Alkaloids		
	Dragendroff's test	+
	Mayer's test	+
	Wagner's test	+
	Hager's test	+
Carbohydrates		
	Fehling's test	+
	Benedict's test	+
Tannins		
	Ferric chloride test	-
	Shinoda test	+
	Alkaline reagent test	+
	Lead acetate solution test	-
Proteins		
	Biuret test	+
	Million's test	+
	Xanthoprotein test	+
	Ninhydrine test	+
Glycosides		
	Keller-killiani test	+
	Bromine water test	+
	Legal's test	
Amino acids		
	Ninhydrine test	+
	Test for tyrosine	+
Terpenes	+	
Flavonoids		+
	Lead acetate	+

Where, (+) = present, (-) = absent

Leaves exhibited analkaloid, flavonoids, glycosides, amino acids as chemical constituents. It also exhibited for tannins in some tests.

Evaluation of hypnotic activity

Thiopental sodium-induced sleeping time

In screening of hypnotic effect, the rats were divided in 4 groups. Group 1(control) was administered normal saline only. Group 2 served as standard that was fed with standard drug- Diazepam. Group 3 and Group 4 were kept as test 1 and test 2 that administered methanolic leaves extract of *A. obesum* at the dose of 200mg/kg and 400mg/kg, respectively. Finally, all the results were compared with the control and standard group for potential response.

This model was tested in rodents to determine how well it could predict when sleep would begin and how long it would last (in minutes). The estimated time it took for sleep to set in was 6.41 0.20* in the control group and 3.42 0.31** in the Diazepam treatment group, while the estimated time spent asleep was 81 1.24** and 178 4.31**, respectively. At a lower dose of 200mg/kg, the methanolic leaves extract of A. obesum showed sleep onset of 5.14 0.12*** and sleep duration (min) of 129 1.23***. However, both sleep onset and duration were considerably reduced by A. obesum methanolic leaf extract. It showed that sleep onset was 4.73 0.29*** and sleep duration was 1612.23*** at a dose of 400mg/kg. When the effects of several extracts were evaluated, ethanolic extract was shown to have the most promise, particularly at larger doses.

When compared to the placebo group, the methanolic leaves extract of A. obesum showed hypnotic potential across the board. The impact at 400mg/kg in the methanolic extract was comparable to the gold standard.

Table 2: Thiopental sodium-induced sleeping time in *A. obesum* treated rodents.

Group	Effects on sleep	
	Sleep onset (min)	Sleep duration (min)
Control (Vehicle)	6.41± 0.20*	81±1.24**
Diazepam (4mg/kg)	3.42± 0.31**	178± 4.31**

Methanolic leaves extract of *A. obesum*(200mg/kg)	5.14± 0.12***	129±1.23***
Methanolic leaves extract of *A. obesum*(400mg/kg)	4.73± 0.29***	161±2.23***

At P<0.05 significance level was considered; n=6
Values were shown in Mean±SEM

Motor co-ordination test

In determination of hypnotic effect, the rats were divided in 4 groups. Group 1 (control) was administered normal saline only. Group 2 served as standard that was fed with standard drug- Diazepam. Group 3 and Group 4 were kept as test 1 and test 2 that administered methanolic leaves extract of *A. obesum* at the dose of 200mg/kg and 400mg/kg, respectively. Finally, all the results were compared with the control and standard group for potential response.

Animals' motor coordination was measured using a rota-rod. In the control group, motor coordination was timed at 118±3.41** seconds, while in the standard group, it was timed at 27±2.11** seconds. While A. obesum methanolic leaf extract showed activity as 76±3.52** at 200mg/kg and 44±1.25*** at 400mg/kg, respectively. Herbal extracts have been proven to improve motor coordination by 101±1.83** at 80mg/kg and 56±.28** at 120mg/kg in various trials. Maximum inhibition was observed in extract at the highest dose when tested against all other treated groups.

When compared to the gold standard medicine Diazepam, however, *A. obesum* showed that it is an effective hypnotic agent.

Table 3: Motor Co-ordination test.

Treatment	Rota-rod movement (sec)
Control (Vehicle)	118± 3.41**
Diazepam (4mg/kg)	27± 2.11**
Methanolic leaves extract of *A. obesum*(200mg/kg)	76± 3.52**
Methanolic leaves extract of *A. obesum*(400mg/kg)	44± 1.25***

At P<0.05 significance level was considered; n=6
Values were shown in Mean± SEM

However, the % inhibition confirms the hypnotic effectiveness. In comparison to other groups, methanolic extract at higher dose following standard showed the highest percentage of inhibition.

Table 4: % Inhibition in Rota-rod test.

Treatment	% Inhibition
Control (Vehicle)	0.00
Diazepam (4mg/kg)	74.67
Methanolic leaves extract of *A. obesum*(200mg/kg)	57.34
Methanolic leaves extract of *A. obesum*(400mg/kg)	71.34

At P<0.05 significance level was considered; n=6
Values were shown in Mean± SEM

Light-dark arena model

In order to evaluate the hypnotic effect, the rats were divided in 4 groups. Group 1 (control) was administered normal saline only. Group 2 served as standard that was fed with standard drug- Diazepam. Group 3 and Group 4 were kept as test 1 and test 2 that administered methanolic leaves extract of *A. obesum* at the dose of 200mg/kg and 400mg/kg, respectively. Finally, all the results were compared with the control and standard group for potential response.

Control group shown the lowest no. of entries 5.64±0.12* and % of time spent as 23.58±0.14*%. The standard group had the highest percentage of time spent (54.61±0.19**) in the light arena (9.29±0.26** entries, 154.20±0.54 sec). At a dose of 200mg/kg, A. obesum leaf methanolic extract enhanced both the number of entries (6.75±0.12***) and the amount of time spent in the light arena (94.29±0.60). Herbal extracts at a dose of 200mg/kg showed 8.32±0.19* (number of entries) and 134±0.48 (time spent) in the light arena.

Table 5: Light/dark arena test.

Treatment	No. of entries (light arena)	Time spent (light arena) (s)	% of time spent (light arena) (s)
Control (Vehicle)	5.64±0.12*	67.36± 0.12	23.58±0.14*
Diazepam (4mg/kg)	9.29±0.26**	154.20± 0.54	54.61±0.19**
Methanolic leaves extract of *A. obesum*(200mg/kg)	6.75±0.12***	94.29±0.60	33.24±0.58**
Methanolic leaves extract of *A. obesum*(400mg/kg)	8.27±14***	136.32±0.17	37.27±0.27***

At P<0.05 significance level was considered; n=6
Values were shown in Mean± SEM

Locomotor activity

The rats were divided in 4 groups. Group 1 (control) was administered normal saline only. Group 2 served as standard that was fed with standard drug- Diazepam. Group 3 and Group 4 were kept as test 1 and test 2 that administered methanolic leaves extract of *A. obesum* at the dose of 200mg/kg and 400mg/kg, respectively. Finally, all the results were compared with the control and standard group for potential response.

The control group had the most locomotor activity (158±0.26**) after 10 minutes, whereas the diazepam-treated group had the lowest (930.24**). At 200mg/kg, A. obesum leaf methanolic extract caused an activity score of 134±0.25*** in rats; at 400mg/kg, the score dropped to 107±0.31***. When compared to methanolic and aqueous extracts, it showed the strongest inhibition in earlier research. Both doses of Adenium obesum showed considerable hypnotic action.

Table 6: Locomotion activity score.

Treatment	Locomotor activity score (Sec ± SEM)
Control (Vehicle)	158±0.26**
Diazepam (4mg/kg)	93±0.24**
Methanolic leaves extract of *A. obesum* (200mg/kg)	134±0.25***
Methanolic leaves extract of *A. obesum* (400mg/kg)	107±0.31***

Significance level was represented by *; P<0.05
n=6; readings were given in Mean± SEM

The release of GABA, an inhibitory neurotransmitter, could be increased, leading to more neurotransmitter inhibition. Because of this, the concentration of chloride ions in the solution would rise, leading to hyperpolarization. This research proves that flowers have hypnotic properties. Diazepam is used to treat insomnia and other sleep disorders because it acts as a central nervous system depressant by binding to the GABAA ionophore complex in the brain. The consequence is calming because activity and excitement are lowered.

The drug utilised as the gold standard in this study, diazepam, has been proven to prolong the duration of barbiturate-induced sleep and decrease the desire to participate in exploratory behaviour. Adenium obesum extract oral dosages of 80 and 120 mg/kg produced a hypnotic effect similar to that of 2 mg/kg diazepam. Popular benzodiazepine diazepam is used to alleviate anxiety and also has strong sedative and hypnotic properties. The actophotometer readings specifically lowered in proportion to the dosage of Adenium obesum extract administered. It is commonly acknowledged that brain activation, which manifests as a central neuronal excitation involving a variety of neurochemical pathways and an increase in cerebral metabolism, is the cause of locomotor activity. It is likely that this channel causes the hypnotic action of both the methanolic and aqueous extract of Adenium obesum because GABAergic transmission can make mice deeply sleepy. The central nervous system (CNS) is depressed as a result of hyperpolarization of the membrane, which is enabled by the opening of chloride channels due to GABA's inhibitory activity and results in hypnotic and sedative effects.

In results, it was found that *A.obesum*methanolic leaves extract is very significant in the preclinical treatment of insomnia. Its mode of action might involve inhibiting neurotransmitter release and encouraging catecholamine breakdown. Additionally, it lowers biogenic amine levels, which has the opposite effect of making you sleepy.

CONCLUSION

In conclusion, *Adenium obesum's* methanolic leaf extract is effective in reducing insomnia and resetting the mind to promote sleep. However, its method of action must be understood in order to choose the right medication for various elements of mental diseases.

It suggests conducting clinical research to assess the hypnotic effects of *A. obesum*, which would have been utilized to treat insomnia and other associated issues. It is of herbal origin, making it readily available and

reasonably priced. Additionally, it advises separating the important active ingredient for the activity and converting it into a proper dosage for better absorption and toleration.

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CONFLICT OF INTEREST
None.

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