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Research Article

Phytochemical screening and anti-obesity potential of aqueous leaves extract of *Adenium obesum* in Wistar rats

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ABSTRACT

The study was based on the phytochemical screening and anti-obesity potential of aqueous leaves extract of *Adenium obesum* in Wistar rats. The fresh leaves of *Adenium obesum* were collected from Bareilly region and were authenticated by the botanist. In a beaker, the powdered leaves of *Adenium obesum* (50g) was soaked into distilled water (1500 ml) for 15 days with gradual stirrings. Later, it was filtered using cotton plug and finally with Whatman filter paper. The obtained slurry was made evaporated/dried using rotatory evaporator. Thus, the extract was found in dried powder form and kept in desiccator to prevent from moisture. Preliminary phytochemical screening was performed for the aqueous leaves extract of *A. obesum*. Handling of the Wistar rats was performed as per the Institutional Animal Ethics Committee. The animals were kept in good conditions, with a 12-hour light-dark cycle and temperature of 25 ± 1 °C. All the rats were divided into 5 groups (n=6) and treated for 30 days i.e., group 1 (control) administered normal saline, group 2 (disease control) administered progesterone, group 3 (std.) administered progesterone + Orlistat (10mg/kg), group 4 (Test 1) administered progesterone + aqueous leaves extract of *A. obesum* (ALAO) (200mg/kg), group 5 (Test 2) administered progesterone + aqueous leaves extract of *A. obesum* (ALAO) (400mg/kg). Parameters i.e., body weight, organs weight, total cholesterol level, food and blood sugar level were evaluated. In results, aqueous leaves extract of *Adenium obesum* significantly exhibited anti-obesity potential by showing the decreased body weight, body organs, total cholesterol level, and blood sugar level. It is also associated with regulating total cholesterol, triglycerides, and organ weights. It concluded that *Adenium obesum* reduced the body weights of rats. Upon examination of the body organs, a reduction in the weights of the liver, spleen, and kidneys was also noted. This study indicates that *Adenium obesum* may be utilized as a recognized treatment for obesity management and the reduction of detrimental cholesterol, hence promoting improved health and longevity, pending more successful research.

Keywords: Anti-obesity, *Adenium obesum*, Orlistat, Blood sugar level, Organ's weight.

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INTRODUCTION

Obesity is a medical disease that refers excessive bodily fat storage that poses a risk to human health. The higher incidence of type II diabetes mellitus is associated with obesity. One early sign of type II diabetes mellitus is impaired glucose tolerance brought on by insulin resistance (Wabitsch et al. 2004). Obesity, according to the WHO, is defined as an excessive buildup of body fat that may have an impact on physiology. Globally, the prevalence of obesity has almost tripled since 1975. In 2016, it was discovered that over 1.9 billion adults were fat. Over 39 million children aged ≤ 5 years were evaluated for being overweight in 2020. It is possible to prevent obesity (WHO, 2021). Obesity must be recognized and diagnosed as soon as possible. AI is a promising substitute for early predictions of obesity and overweight risk since it can provide prompt, accurate, and instantaneous responses (Safaei et al. 2021).

According to Withrow and Alter (2011), these increasing numbers highlight the need for a suitable intervention to control obesity and lessen the future healthcare burden associated with comorbidities like hypertension, dyslipidemia, type 2 diabetes, coronary heart disease, stroke, sleep apnea, and certain types of cancer. Three methods are used to treat obesity: medication, surgery, and behavioral and lifestyle changes. Changes in behavior and lifestyle are only effective as a preventive measure, and they are hard to implement in obese individuals (Apovian, 2016). There aren't many anti-obesity medications on the market, and the ones that are available have poor effectiveness or unavoidable adverse effects that restrict their use. Although bariatric surgery and other surgical procedures are effective, weight loss following surgery cannot be avoided. This necessitated the adoption of safe, effective natural anti-obesity drugs to prevent weight gain and the comorbidities that follow (Finer, 2021).

Plant profile

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. Native to semi-arid, sub-Saharan regions of eastern and southwestern Africa as well as the Arabian Peninsula, *Adenium obesum*, sometimes known as the desert rose, is a perennial succulent with thick stems and sparse leaves. This plant will eventually grow to a height of 6 to 9 feet in the wild, but it will be considerably smaller in containers. It produces twisted, leafless, gray-green to brown branches from a large, bloated, and frequently ugly caudex that is typically partially buried underground. At the extremities of the stems are leathery, spirally organized, fleshy, oval, gray-green leaves that can grow up to 4 inches long. The salverform blooms are narrow, bell-shaped, and up to 2" across. They have five projecting stamens and spreading lobes, and they can be rose, pink, or red with white to pale yellow throats. In the wild, some flowers bloom irregularly throughout the year, with the majority blooming in the summer. Flowers usually only bloom in

the summer when houseplants are used. The sap of milky plants is toxic.

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).



Fig 1. *Adenium obesum* plant

Taxonomy

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

Species : *obesum*

The chemical components betulin and rosmarinic acid were visible in the stem and bark. 5,7,3,4-Tetrahydroxy flavone and 3,5,7,3,4,5-Hexahydroxy flavone were found in the stem, while the leaves indicated the presence of several chemical ingredients, including Honghelin and Obeside-B&C. (Hossain et al. 2013). The present research focused on the phytochemical screening and anti-obesity potential of aqueous leaves extract of *Adenium obesum* in Wistar rats.

MATERIALS AND METHODS

Chemicals and apparatus

Fresh leaves of *Adenium obesum*, progesterone, distilled water, Wistar rats, rotatory evaporator, weighing machine and ethanol.

Collection and authentication of plant

The fresh leaves of *Adenium obesum* were collected from Bareilly region and were authenticated by the botanist. The leaves were washed to make these dust-free; dried in shade. After, dried leaves were crushed and rendered into fine powder form.

Preparation of the extract

In a beaker, the powdered leaves of *Adenium obesum* (50g) was soaked into distilled water (1500 ml) for 15 days with gradual stirrings. Later, it was filtered using cotton plug and finally with Whatman filter paper. The obtained slurry was made evaporated/dried using rotatory evaporator. Thus, the extract was found in dried powder form and kept in desiccator to prevent from moisture (Khan et al. 2020).

Preliminary phytochemical screening

Various phytochemicals were detected using following procedures:

Alkaloids Detection

Separately, filtered extract solutions in diluted HCl were made. Filtrates were subjected to the Mayer's reagent, potassium mercuric iodide, as part of the Mayer's test. The development of a yellow precipitate indicates the presence of alkaloids.

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.

Detection of Glycosides

Fehling's test: Fehling's solutions A and B were heated for one minute while diluted with distilled water. This clear blue solution was mixed with eight drops of plant extract. After that, it was boiled in a water bath for five minutes while mixed with one milliliter of Fehling's solution. The presence of glycosides is indicated by brick-red precipitation.

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

In order to prepare 0.5 grams of the dried powdered sample for the ferric chloride test, it was boiled in a test tube with 20 milliliters of water. When a few drops of 0.1% FeCl₃ were added, the hue changed to either blue-black or brownish green-black.

Two milliliters of plant extract and two milliliters of water were combined for the lead acetate analysis. After that, this mixture was added to 0.01g of lead acetate and mixed briskly. When white turbidity and precipitate occur, tanning chemicals are present.

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

Molisch'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.

Preparation of animals

Handling of the Wistar rats was performed as per the Institutional Animal Ethics Committee. The animals were kept in good conditions, with a 12-hour light-dark cycle and temperature of 25±1 °C. They received a conventional rat pellet diet and unlimited amounts of water while the relative humidity was kept between 44 and 56 percent. Rats were taken off their food an hour before the experiment (Bhajoni et al. 2016).

Group design

All the rats were divided into 5 groups (n=6) and treated for 30 days as followings-

Group 1 (control) administered normal saline each day.

Group 2 (disease control) administered progesterone.

Group 3 (std.) administered progesterone + Orlistat (10mg/kg).

Group 4 (Test 1) administered progesterone + aqueous leaves extract of *A. obesum* (ALAO) (200mg/kg).

Group 5 (Test 2) administered progesterone + aqueous leaves extract of *A. obesum* (ALAO) (400mg/kg).

Pharmacological evaluation of anti-obesity potential

Body weight

Prior to the medicine being administered and following the completion of the dosage, the weight of each animal is measured. Body weight is compared before and after the medication is administered.

Blood sugar level

Seven blood glucose readings are taken at 0, 5, 10, and 15 days following the start of medication dosage. A blood glucometer manufactured by Dr. Morepen is used to estimate blood glucose levels after a blood sample is drawn via the puncturing tail vein. It is a simple and genuine process (Jung et al. 2021).

Weight of organs

After being sacrificed, all of the rats from the various groups are burned. Organs such the brain, kidney, and lever were weighed independently to verify the effect on other organs as well.

Total Cholesterol level

The lipid profile test was carried out correctly and in accordance with the guidelines. Every test was carried out using a particular medical kit that was sold on the

market. Using a medical kit purchased from a certified manufacturer and supplier, the total cholesterol level in blood plasma was also determined (Dholi et al. 2011).

Statistical analysis

ANOVA was followed by a two-tailed T test to evaluate the statistical results. To do the statistical analysis, values were displayed as S.E.M. Graph Pad Prism. The results were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Percentage yield

The aqueous leaves extract of *A. obesum* showed the percentage yield of 47.38 % based on practical yield.

Preliminary phytochemical screening

The below table shows the Phytoconstituents of aqueous leaves extract of *A. obesum*:

Table 1. Phytoconstituents of aqueous leaves extract of *A. obesum*

S. N.	Phytochemicals	Aqueous leaves extract of <i>A. obesum</i>
1.	Alkaloids	+++
2.	Saponins	++
3.	Carbohydrates	++
4.	Tannins	=
5.	Glycosides	++
6.	Amino acids	+
7.	Terpenoids	++
8.	Flavonoids	++
9.	Cardiac glycosides	+
10.	Pregnanes	++

+ = Presence; ++ = Moderate; +++ = Abundance

Pharmacological evaluation of anti-obesity potential

Body weight

Progesterone (10mg/kg) + ALAO (200mg/kg) and Progesterone (10mg/kg) + ALAO (400mg/kg) treated rats showed the body weight as 149.62 ± 0.24 g and 144.19 ± 0.20 g, respectively which represents the decreasing weight of rats in dose-dependent manner. It was significant when compared with the normal control group. Therefore, this maintained body weights of rats while given with high fat diet.

Table 1. Body weight determination of *A. obesum* treated rats

Treatment	Body weight (g)	
	Before	after
Normal Saline	120.45 ± 0.26	139.23 ± 0.35
Progesterone (10mg/kg)	124.21 ± 0.14	158.31 ± 0.19
Progesterone (10mg/kg) + Orlistat (10mg/kg)	118.14 ± 0.34	143.56 ± 0.27
Progesterone (10mg/kg) + ALAO (200mg/kg)	126.23 ± 0.30	149.62 ± 0.24
Progesterone (10mg/kg) + ALAO (400mg/kg)	122.16 ± 0.28	144.19 ± 0.20

Estimation of blood glucose level

Blood Glucose Level was estimated in day 1, day 3, day 5, day 10 and day 15 in all the treatment group of animals. Blood Glucose Level was estimated in Progesterone (10mg/kg) + ALAO (400mg/kg) treated rats as 104.46 ± 0.25 mg/dl and 109.45 ± 0.22 mg/dl at the day 5 and day 15, respectively. However, At day 5 and day 10, the blood glucose level was estimated as 113.45 ± 0.59 mg/dl and 129.21 ± 0.16 mg/dl, respectively in the progesterone (10mg/kg) treated rats. At both the doses *A. obesum* showed anti-obesity potential in terms of lowering the blood sugar level in rats.

Table 6. Estimation of blood sugar level of *A. obesum* treated rats

Treatment	Blood sugar level (mg/dl)				
	Day 1	Day 3	Day 5	Day 10	Day 15
Normal Saline	85.56 ± 0.22	82.45 ± 0.28	86.56 ± 0.21	89.34 ± 0.18	84.42 ± 0.27
Progesterone (10mg/kg)	86.32 ± 0.15	91.33 ± 0.20	102.45 ± 0.29	113.45 ± 0.59	129.21 ± 0.16
Progesterone (10mg/kg) + Orlistat (10mg/kg)	84.51 ± 0.11	89.26 ± 0.13	94.29 ± 0.18	98.34 ± 0.20	102.30 ± 0.26
Progesterone (10mg/kg) + ALAO (200mg/kg)	86.45 ± 0.19	91.56 ± 0.43	96.71 ± 0.34	106.18 ± 0.38	112.62 ± 0.39
Progesterone (10mg/kg) + ALAO (400mg/kg)	85.26 ± 0.12	90.26 ± 0.26	94.58 ± 0.26	104.46 ± 0.25	109.45 ± 0.22

Weight of organs

Rats were anesthetized with diethyl ether. Then they were sacrificed with cervical dislocation method, prior dissecting different organs to weight. Progesterone (10mg/kg) + ALAO (200mg/kg) and Progesterone

(10mg/kg) + ALAO (400mg/kg) showed the weight of liver as 6.32 ± 0.45 g and 6.11 ± 0.34 g, respectively. It was found effectively significant at lower and higher doses when compared with the control.

Table 2. Determination of weight of organs of *A. obesum* treated rats

Treatment	Spleen (g)	Kidney (g)	Liver (g)
Normal Saline	0.64 ± 0.19	1.32 ± 0.14	4.45 ± 0.56
Progesterone (10mg/kg)	0.82 ± 0.25	1.92 ± 0.26	6.67 ± 0.22
Progesterone (10mg/kg) + Orlistat (10mg/kg)	0.68 ± 0.29	1.49 ± 0.20	5.54 ± 0.31
Progesterone (10mg/kg) + ALAO (200mg/kg)	0.78 ± 0.45	1.62 ± 0.17	6.32 ± 0.45
Progesterone (10mg/kg) + ALAO (400mg/kg)	0.73 ± 0.25	1.53 ± 0.35	6.11 ± 0.34

Total cholesterol level

Adenium obesum significantly lowered the total cholesterol level when estimated and compared with the

control and disease control groups. It showed increasing effect with the increase in the time (day 1 to day 15).

Table 3. Estimation of Total Cholesterol (TC) of *A. obesum* treated rats

Treatment	TC (mg/dl)				
	Day 1	Day 3	Day 5	Day 10	Day 15
Normal Saline	44.19 ± 0.34	47.67 ± 0.23	51.45 ± 0.21	53.12 ± 0.20	55.51 ± 0.59
Progesterone (10mg/kg)	46.62 ± 0.57	51.67 ± 0.45	57.27 ± 0.38	68.19 ± 0.75	79.25 ± 0.34
Progesterone (10mg/kg) + Orlistat (10mg/kg)	45.34 ± 0.11	49.29 ± 0.26	52.40 ± 0.14	55.26 ± 0.29	58.87 ± 0.20
Progesterone (10mg/kg) + ALAO (200mg/kg)	43.37 ± 0.24	48.65 ± 0.20	54.56 ± 0.18	63.54 ± 0.10	68.29 ± 0.19
Progesterone (10mg/kg) + ALAO (400mg/kg)	44.27 ± 0.45	49.33 ± 0.14	53.23 ± 0.78	57.24 ± 0.10	65.29 ± 0.19

The decrease in protein and glucose levels in blood plasma suggests the anti-obesity efficacy of the aqueous leaf extract of *Adenium obesum*. It verifies that insulin regulation normalizes or enhances the sensitivity of Tyrosine Kinase receptor subtypes, facilitating improved binding and activation of glucose transporters. Consequently, it promotes the liberation of glucose molecules for enhanced distribution to targeted organs and generates ATP for optimal metabolic cycles of tissues. Its mechanism may involve receptor sensitization in Type 2 diabetes mellitus or insulin secretion in Type 1 diabetes mellitus. The aqueous leaf extract of *Adenium obesum* demonstrated considerable modulation to validate its anti-obesity potential. It reduced the body weights of rodents relative to the control at both doses of 200 mg/kg and 400 mg/kg. The weights of the spleen, liver, and kidneys were also reduced when measured. Obesity is associated with a heightened incidence of type II diabetes mellitus. Glucose tolerance impairment due to insulin resistance is regarded as an initial indicator of type II diabetes mellitus. We assessed insulin resistance and glucose metabolism dysfunction by measuring fasting blood glucose and serum insulin levels, subsequently calculating the HOMA-IR ratio in experimental mice. A notable reduction in fasting blood glucose levels, serum insulin, and consequently the HOMA-IR index indicated that the treatments of the formulations contributed to the normalization of impaired glucose metabolism and perhaps mitigated insulin resistance in diet-induced obese rats, suggesting their prospective antidiabetic efficacy. The decrease in

fat droplets during histological assessment of the liver and the reduction in adipocyte size in white adipose tissue further corroborate enhanced fat accumulation in participants subjected to a high-fat diet. The lipid profile is disturbed in obese individuals, increasing their susceptibility to cardiovascular illnesses. Timely management of lipid profiles and obesity is thought to avert life-threatening cardiovascular issues in adults (Mansour et al. 2016). Our assessment of the lipid profile indicated that formulation A markedly reduced cholesterol, triglycerides, and LDL/VLDL, while elevating HDL levels. Formulation A had a reduced atherogenic index while delivering 38.7% cardioprotection. A notable decrease in LDL/VLDL levels and the atherogenic index was also found with formulation B treatment, resulting in 7.12% cardioprotection.

In results, aqueous leaves extract of *Adenium obesum* significantly exhibited anti-obesity potential by showing the decreased body weight, body organs, total cholesterol level, and blood sugar level. It is also associated with regulating total cholesterol, triglycerides, and organ weights.

CONCLUSION

It concluded that the reduction in body weight is a significant indicator of anti-obesity efficacy; *Adenium obesum* effectively reduced the body weights of rats. Upon examination of the body organs, a reduction in the weights of the liver, spleen, and kidneys was also noted. This activity may result from reducing energy levels, hence regulating weight.

This study indicates that *Adenium obesum* may be utilized as a recognized treatment for obesity management and the reduction of detrimental cholesterol, hence promoting improved health and longevity, pending more successful research.

CONFLICT OF INTEREST

Authors have declared for none conflict of interest.

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