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EVALUATION OF ANTIOXIDANT AND ANTIDIABETIC POTENTIAL OF LEAVES EXTRACT OF *ADENIUM OBESUM* IN ALLOXAN-INDUCED DIABETIC RATS

***[1]Rahul Sharma, [2]Shamim Ahmad and [3]Jainedra Kumar**

[1]Research Scholar, Translam Institute of Pharmaceutical Education and Research, Meerut, Uttar Pradesh.

[2]Professor & Director, Translam Institute of Pharmaceutical Education and Research, Meerut, Uttar Pradesh.

[3]Assistant Professor, Translam Institute of Pharmaceutical Education and Research, Meerut, Uttar Pradesh.

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Corresponding Author: Rahul Sharma

Address: Research Scholar, Translam Institute of Pharmaceutical Education and Research, Meerut, Uttar Pradesh.

Email ID: rahulsharmabsr833@gmail.com

ABSTRACT

Herbal remedies are thought to have been used for the ancient period. Herbal remedies have been used in countries all over the world since 2800BC. The present research was based on the evaluation of antioxidant and antidiabetic potential of leaves extract of *Adenium obesum* in alloxan-induced diabetic rats. The fresh leaves of *Adenium obesum* were collected from Meerut region, UP and were authenticated from a botanist. The leaves were dried at 40ºC temp. after being cleaned to remove any dust. Weighed out powder was steeped in ethanol solvent for 15 days, stirring gradually during that time. A rotary evaporator was used to partially vacuum-dry the mixed slurry that was produced. We received 130-150g Wistar rats of both sexes from the Animal House at the Translam Institute of Pharmaceutical Education and Research in Meerut. The animals were kept under ideal circumstances, with a 12-hour light/dark cycle and room temperatures of 25±1°C. The rats are then given a single intraperitoneal injection of freshly manufactured alloxan monohydrate solution (150 mg/kg, s. c.), which causes them to become diabetic. All the animals were kept in different 5 groups; n=6 and treated for 21 days i.e., group 1 given normal saline, group 2 given alloxan

(150mg/kg, s. c.), group 3 given alloxan (150mg/kg, s. c.) + Glibenclamide (5mg/kg), group 4 given alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (200mg/kg, p. o.) and group 5 given alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (400mg/kg, p. o.). Evaluation of antioxidant and antidiabetic potential was done through parameters i.e., estimation of DPPH free radical scavenging/antioxidant Assay, body weight, blood glucose level and oral glucose tolerance test. In all the treated groups, the blood sugar level was estimated after 0, 5, 10, 15 and 21 days. After 21 days, Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (200mg/kg, p. o.) and Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (400mg/kg, p. o.). treated rats showed the blood glucose level as 109.23±0.46mg/dl and 98.46±0.24mg/dl, respectively. The research suggests that *Adenium obesum* is an effective herb in the management of diabetes but it's mode of action is still unknown. So further research may carried-out to confirm its mode of action and optimum doses required in management of DM.

KEYWORDS: *Adenium obesum*, antidiabetic, alloxan, insulin, antioxidant.

INTRODUCTION

Herbal remedies are thought to have been used for the ancient period. Herbal remedies have been used in countries all over the world since 2800BC.[1] Plants possess significant potential in the treatment and management of diseases. Traditional healers in numerous nations have utilized diverse botanical species to address a wide range of ailments.[2] In 1922, Banting et al. extracted and refined the insulin from bovine pancreas at the University of Toronto, causing the development of a viable therapy for diabetes.[3][4] Type 1 diabetes usually affects kids or teenagers, while type 2 diabetes mellitus usually affects middle-aged and older adults who have had high blood sugar levels for an extended period of time due to poor food and lifestyle choices.[4] Because Type 1 and Type 2 have quite different aetiologies, there are differences in their causes, symptoms, and therapy approaches.[5] Roughly 45% of youngsters exhibit symptoms prior to reaching the age of ten. The incidence rate among individuals aged below 20 is approximately 2.3 per 1000. Among certain populations, particularly older European males aged 13 and above, there is a higher likelihood of developing T1DM compared to females.[6][7] The prevalence of Type 1 DM has been steadily rising on a global scale. Annually, rates in Europe and Middle East are experiencing a steady increase of 2% to 5%.[8] For instance, the prevalence of Type 2 T2DM is significantly lower among Pima

Indians in Mexico as compared to P. Indians in the US with rates of 6.9% and 38% respectively.[9][10]

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 home to the desert rose since it is a country with a diverse range of flora. Many different diseases are treated with parts of a particular species.[11][12] Because of their potential medical benefits, a number of plant species have been selected for commercial cultivation.[13] Betulin and rosmarinic acid were the chemical components that were visible in the stem and bark. While leaves proved the presence of several chemical ingredients, including Honghelin, Obeside-B & C, the stem displayed flavones.[14][15] The present research was based on the evaluation of antioxidant and antidiabetic potential of leaves extract of *Adenium obesum* in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Experimental requirements

Adenium obesum leaves, Metformin, antioxidant kit, 1,1′-diphenyl-1-picrylhydrazyl, distilled water, water bath evaporator, weight machine, and ethanol.

Collection, authentication, and extraction of the plant

The fresh leaves of *Adenium obesum* were collected from Meerut region, UP and were authenticated from a botanist. The leaves were dried at 40ºC temp. after being cleaned to remove any dust. The dehydrated leaves were ground into first coarse and then fine powders. Weighed out powder was steeped in ethanol solvent for 15 days, stirring gradually during that time. A rotary evaporator was used to partially vacuum-dry the mixed slurry that was produced. The percentage yield of the extract of the *Adenium obesum* extract was calculated thru below mentioned formula[16]

$$\text{percent yield} = \frac{\text{actual yield}}{\text{theoretical yield}} \times 100\%$$

Phytochemical screening

Various phytoconstituents were looked for in the plant extracts.[17]

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: Fehling's solutions A and B with distilled water dilution were heated for a minute. Eight drops of plant extract were added to this clear blue mixture. It was then cooked in a water bath for five minutes together with one millilitre of Fehling's solution. Precipitation that is brick red indicates the presence of glycosides.

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

Concentrated H2SO4 and 2 milliliters of chloroform were combined with 5 milliliters of aqueous plant crude extract. The basal chloroform layer became red, suggesting the presence of steroids.

Detection of carbohydrates

Molish's Test

To 2-3 ml of extract from each solvent, add a few drops of an alcohol-naphthol solution, shake, and then pour concentrated H2SO4 from the test tube's sides. Where the two liquids converge, a violet ring is seen.

Fehling 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 (mL) 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.

Preparation of animals

We received 130-150g Wistar rats of both sexes from the Animal House at the Translam Institute of Pharmaceutical Education and Research in Meerut. The animals were kept under ideal circumstances, with a 12-hour light/dark cycle and room temperatures of 25±1°C. They were fed a regular mouse pellet diet and given access to unlimited water, with the relative humidity being kept at 44-56%. One hour before the trial, the rats were not fed.

Induction of diabetes mellitus

After a 12- to 14-hour fast, the weight and fasting blood glucose level of the Wistar rats are measured using a glucometer. The rats are then given a single intraperitoneal injection of freshly manufactured alloxan monohydrate solution (150 mg/kg, s. c.), which causes them to become diabetic. Before being injected, alloxan was produced by weighing each animal individually and solubilizing it with 0.5 millilitre of sodium citrate at pH 4.5. The animals were given food and drink thirty minutes after the alloxan was given to them. Each animal's plasma blood glucose level was measured 48 hours after receiving an alloxan injection by drawing blood from the tail; animals that had fasting blood glucose levels greater than 200 mg/dl were included in the study.[18]

4.6 Group design

All the animals were kept in different 5 groups; n=6 and treated for 21 days[19] as follows-

Group 1: rats given normal saline.

Group 2: rats given alloxan (150mg/kg, s. c.).

Group 3: rats given alloxan (150mg/kg, s. c.) + Glibenclamide (5mg/kg) are given

Group 4: rats given alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (200mg/kg, p. o.).

Group 5: rats given alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (400mg/kg, p. o.).

4.7 Evaluation parameters

4.7.1 Estimation of DPPH Radical Scavenging Assay

Tariq et al.'s approach[20] was used to assess the 1,1′-diphenyl-1-picrylhydrazyl free radical scavenging capability of plant extracts in vitro. The assay involved reacting 1.6ml of 0.135mM DPPH dissolved in 100% v/v methanol with 0.4 mL of different methanol crude extract concentrations (0.0078-2mg/ml). After giving the reaction mixture a good vortex, it was kept at room temperature for 30 minutes in the dark. Mixture's absorbance was measured at 517nm. In this case, the extract concentration required to reduce the DPPH radical absorbance by 50% was computed. The reference medication was rutin, which was utilised at the same conc. as the plant extracts.

4.7.2 Body weight

To guarantee consistent dosing, the animals in each group are weighed both before and after the medication is administered. The weight of the body is recorded both before and after the medication is administered.

4.7.3 Blood sugar level

Seven blood glucose readings are taken at0,5,10, and 15 days following the initiation of medication therapy. Dr. Morepen's blood glucometer is used to estimate blood glucose levels after a blood sample is taken from the puncturing tail vein. This process is genuine and simple.

4.7.4 Oral glucose tolerance test

Following a fortnight of administering the plant extracts, the animals were allowed to fast for 12–14 hours while still having unrestricted access to water, and their blood glucose levels were assessed four times during this period. A 1 mL/kg volume of glucose solution (2g/kg of body weight) was given orally. After the delivery of glucose, blood samples were taken 30, 60, & 120 min later.[21]

RESULTS AND DISCUSSION

Percentage yield

The percentage yield of ethanolic *Adenium obseum* leaves extract of was found to be 63.24% when calculated in the extract obtained through maceration.

Determination of phytochemicals

It showed saponins, alkaloids, flavonoids, and protein in abundance. However, carbohydrates, terpenes, glycosides, and amino acids were found in moderate amount in ethanolic *Adenium obesum* leaves extract**.** Tannins were found absent when observed. Therefore, *Adenium obesum* shown as a rich source of phytochemicals as mentioned in below table:

Table 1: Determination of Phytochemicals.

S. No.	Phytochemicals	Ethanolic *Adenium obseum* leaves extract
1.	Saponins	++
2.	Carbohydrates	+
3.	Alkaloids	++
4.	Tannins	_
5.	Terpenes	+
6.	Glycoside	+

7.	Flavonoid	++
8.	Amino acids	+
9.	Protein	++

Where (+)= Moderate, (++)= Abundance, (-)=Negative

Evaluation of antioxidant and antidiabetic potential

DPPH Radical Scavenging Assay

In this assay, various concentrations i.e., 200µg/ml, 400µg/ml & 800µg/ml were tested for water, herbal extract, and ascorbic acid. The ethanolic *Adenium obesum* leaves extract was compared with std. ascorbic acid.

The ethanolic *Adenium obesum* leaves extract, water and ascorbic acid were demonstrated the DPPH Scavenging assay (% inhibition) as 35.48±0.26%, 17.53±0.24%, and 32.19±0.58% respectively, at the concentration of 200µg/ml. However, at highest conc. 800µg/ml, ethanolic *Adenium obesum* leaves extract, water and ascorbic acid were demonstrated the DPPH Scavenging assay (% inhibition) as 58.26±0.48%, 33.19±0.49% and 98.23±0.39%, respectively. Thus, effect of herbal extract was found statistically significant when compared with water. The ascorbic acid showed highest free radical scavenging potential. The antioxidant action might be due to blockage or suppression of free radical formation.

The % inhibition (DPPH Scavenging assay) was observed as follows-

Table 2. DPPH Free-Radical Scavenging Assay

Concentration	DPPH Scavenging Assay (% Inhibition)		
	Water	Herbal extract	Ascorbic acid
200µg/ml	17.53±0.24	35.48±0.26	32.19±0.58
400µg/ml	22.30±0.64	43.19±0.34	64.35±.46
800µg/ml	33.19±0.49	58.26±0.48	98.23±0.39

Data shown as mean± S.D.

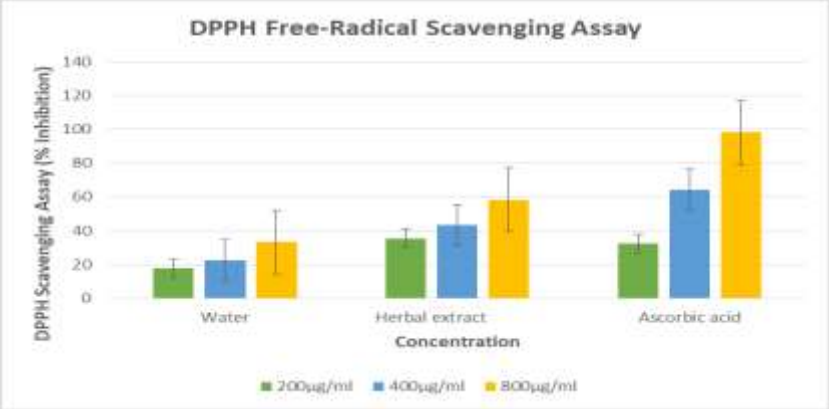

Fig. 1: DPPH Free-Radical Scavenging Assay.

Body weight measurements

The anti-diabetic potential of *Adenium obesum* was evaluated in animals which were divided into 5 groups. The Group 1 was administered normal saline, group 2 administered Alloxan (150mg/kg, s. c), group 3 administered Alloxan (150mg/kg, s. c.) + Glibenclamide (5mg/kg), group 4 administered Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (200mg/kg, p. o.) and group 5 administered Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (200mg/kg, p. o.) once daily for 21 days.

Table 3: Body weight measurements of *Adenium obesum* treated rats.

Treatment	**Body weight (g)**	
	Before	***after***
Normal saline	124.29±0.20	148.25±0.14
Alloxan (150mg/kg, s. c)	129.24±0.36	184.38±0.21
Alloxan (150mg/kg, s. c.) + Glibenclamide (5mg/kg)	132.36±0.49	157.26±0.62
Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (200mg/kg, p. o.)	130.29±0.53	171.43±0.57
Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (400mg/kg, p. o.).	128.47±0.62	163.35±0.47

Significant data at P≤0.05 & n=6

Data demonstrated as Mean±SEM

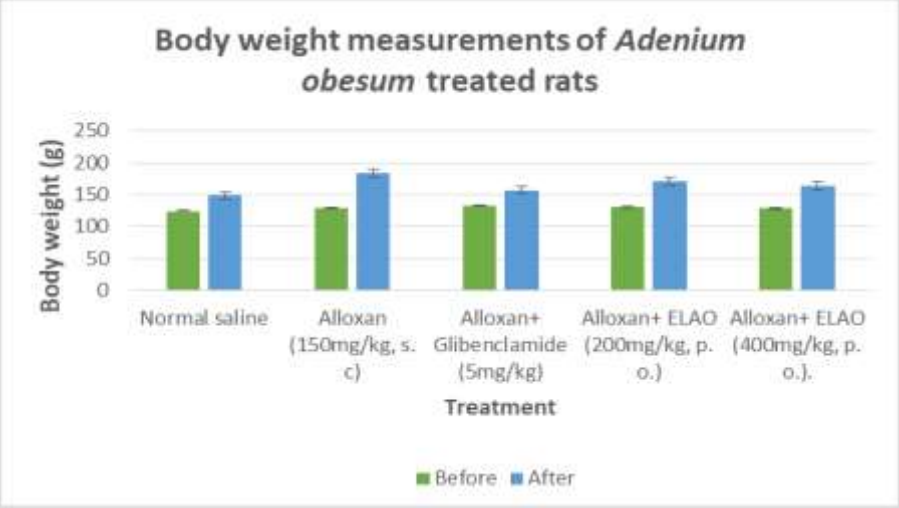

Fig. 2: Graphical data of body weight measurements of *A. obesum* treated rats.

Estimation of blood sugar level

The anti-diabetic potential of *Adenium obesum* was evaluated in animals which were divided into 5 groups. The Group 1 was administered normal saline, group 2 administered Alloxan (150mg/kg, s. c), group 3 administered Alloxan (150mg/kg, s. c.) + Glibenclamide (5mg/kg),

group 4 administered Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (200mg/kg, p. o.) and group 5 administered Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (200mg/kg, p. o.) once daily for 21 days.

In all the treated groups, the blood sugar level was estimated after 0, 5, 10, 15 and 21 days. After 21 days, Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (200mg/kg, p. o.) and Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (400mg/kg, p. o.). treated rats showed the blood glucose level as 109.23±0.46mg/dl and 98.46±0.24mg/dl, respectively. However, in disease control group (Alloxan (150mg/kg, s. c), the blood sugar level was highest 169.24±0.43mg/dl. Therefore, at both the doses *Adenium obesum* showed anti-diabetic action when observed in different parameters estimated.

A decrease in blood plasma and glucose levels is indicative of the herbal extract's hypoglycemic properties. It demonstrates that insulin regulation returns to normal or that tyrosine kinase receptor subtype sensitivity is increased for improved binding and glucose transporter opening. As a result, it helps release glucose molecules for improved distribution to the intended organs and produces ATP energy for healthy tissue metabolism cycles. Its mechanism of action may be dependent on insulin release in Type 1 DM or receptor sensitization in Type 2 DM.

Table 4: Estimation of blood sugar level.

Treatment	Blood sugar level (mg/dl)				
	0 days	5 days	10 days	15 days	21 days
Normal saline	84.14±0.25	85.35±0.25	86.15±0.64	85.45±0.24	86.45±0.25
Alloxan (150mg/kg, s. c)	86.19±0.53	104.24±0.39	142.47±0.56	184.14±0.16	169.24±0.43
Alloxan (150mg/kg, s. c.) + Glibenclamide (5mg/kg)	85.26±0.48	89.45±0.36	93.25±0.44	95.43±0.23	94.42±0.24
Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (200mg/kg, p. o.)	88.16±0.25	90.24±0.31	98.34±0.46	106.16±0.35	109.23±0.46
Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (400mg/kg, p. o.)	85.32±0.62	88.46±0.47	95.26±0.35	99.26±0.19	98.46±0.24

Significant data at P≤0.05 & n=6

Data demonstrated as Mean±SEM

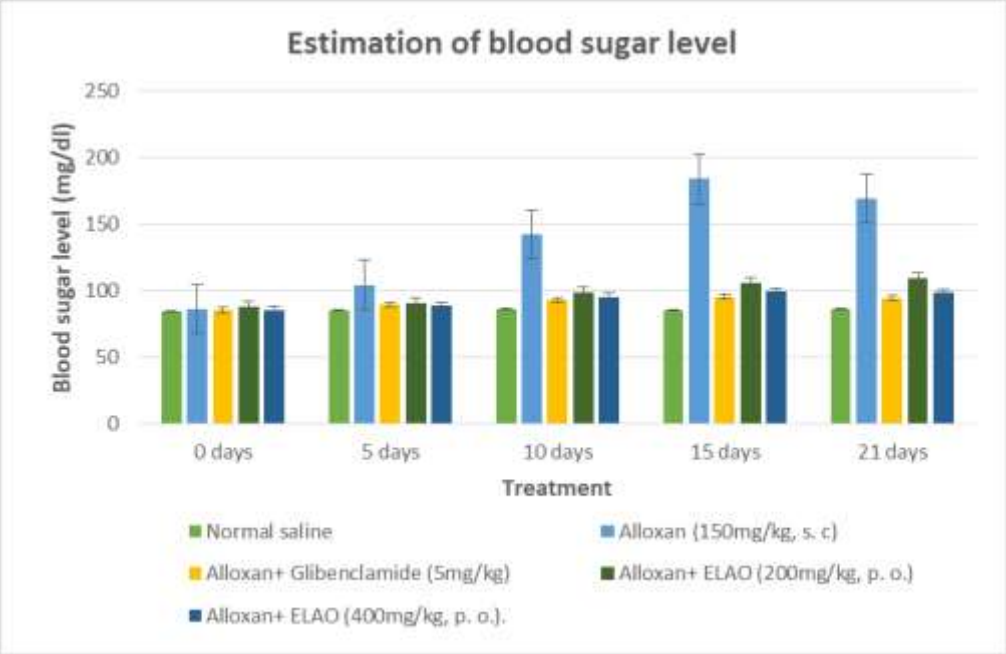

Fig. 3: Graphical data of estimation of blood sugar level.

5.3.4 Oral glucose tolerance test

The hypoglycemic potential of *Adenium obesum* was evaluated in animals which were divided into 5 groups. The Group 1 was administered normal saline, group 2 administered Alloxan (150mg/kg, s. c), group 3 administered Alloxan (150mg/kg, s. c.) + Glibenclamide (5mg/kg), group 4 administered Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (200mg/kg, p. o.) and group 5 administered Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (200mg/kg, p. o.) once daily for 21 days.

In oral glucose tolerance test, the blood sugar level was found increasing with the time i.e., after 60 min, the blood glucose levels were observed highest in all the groups. However, after 120 min it was lowered due to better adaptability and glucose utilization. After 120 min, Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (200mg/kg, p. o.) and Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (400mg/kg, p. o.) treated rats showed blood glucose level as 112.26±0.38mg/dl and 103.24±0.41mg/dl, respectively.

Table 5: Estimation of blood sugar level in oral glucose tolerance test.

Treatment	Blood sugar level (mg/dl) in OGTT		
	30 min	60 min	120 min
Normal saline	86.36±0.20	98.31±0.64	85.26±0.32
Alloxan (150mg/kg, s. c)	179.38±0.49	190.24±0.16	164.12±0.52
Alloxan (150mg/kg, s. c.) + Glibenclamide (5mg/kg)	104.17±0.34	115.35±0.46	96.46±0.63
Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (200mg/kg, p. o.)	118.36±0.24	127.26±0.32	112.26±0.38
Alloxan (150mg/kg, s. c.) + ethanolic leaves extract of *Adenium obesum* (ELAO) (400mg/kg, p. o.)	106.23±0.39	114.18±0.26	103.24±0.41

Significant data at P≤0.05 & n=6

Data demonstrated as Mean±SEM

Furthermore, aside from lowering blood glucose levels, these phytochemicals have been discovered to stimulate the restoration of damaged beta cells and safeguard beta cells from oxidative stress in diabetic rats during experimental trials. A study has found that *Artemisia afra* has hypoglycaemic effect in diabetic rabbits. This activity is attributed to the presence of Saponins, which may stimulate the production of insulin via repairing pancreatic beta cells.[22] Additionally, some of the bioactive components in this study may enhance the activity of glycolytic and glyconeogenic enzymes either synergistically or independently.[23]

CONCLUSION

Furthermore, it was observed that these extracts did not cause any weight loss in diabetic mice. *Adenium obesum* has potential for treatment of DM. Additional research is required to determine the specific mechanism by which this plant exhibits its antidiabetic properties. Ultimately, the antidiabetic response of the leaves extract of *Adenium obesum* may be attributed to its antioxidant action and its ability to enhance the effectiveness of insulin. Confirmation of the mechanism of action requires molecular investigations to determine the specific receptor subtypes it targets and to explore methods for enhancing its binding efficiency.

The research suggests that *Adenium obesum* is an effective herb in the management of diabetes but it’s mode of action is still unknown. So further research may carried-out to confirm its mode of action and optimum doses required in management of DM.

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Nil.

CONFLICT OF INTEREST

Authors declared for none ‘conflict of interest’.

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