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# Histological Changes Of Kidney, Liver, Pancreas In Alloxan Induced Diabetic Mice Treated With Selenicereus Undatus (Haw). Ethanolic Extract

P. Sonu<sup>1</sup>, P. Raja babu, P Naga Chandrika, Gara Abhilash, Prof S. Jithender Kumar Naik, C. Anusha Pawar. P Dharma teja, P Surya teja.

 $^{1,2*,3}$  Department of Zoology, Osmania University Hyderabad, Telangana State-500007 India .

\*Corresponding author: Prof Jithender Kumar Naik, Email: drnaik8777@yahoo.com

# Abstract

Forty-eight swiss albino mice, weighing between 25-30 grams were used in this experiment. There are a total of six mice in each group and each group was divided into eight equal sections. Diabetes was induced in group 2,3,4,5,6,7 using alloxan monohydrate of (150 mg/kg body weight) administered intraperitoneally to overnight fasted animals. Group 1:- Served as the control. Group 2:- Mice induced with diabetes .Group 3:- Diabetic induced mice treated with metformin of 150 mg/kg body weight. Group 4:- Diabetic induced mice treated with Epicarp 45 mg/kg body weight. Group 5:- Diabetic mice treated with Epicarp of 75 mg/kg body weight. Group 6:-Diabetic induced mice treated with Endo mesocarp of 45 mg/kg body weight. Group 7:-Diabetic mice treated with Endo mesocarp of 75 mg/g body weight. Treatments were administered via orogastric intubation for 27 days. All animals had free access to pellet feed and water throughout the experiment. On the 28<sup>th</sup> day, the animals were sacrificed, and the peritoneium was opened to collect the pancreas, kidneys, and liver, which were preserved in 10% formaldehyde for histological observation using the haematoxyline and eosin staining technique. Histological analysis showed regenerative changes in pancreas, liver, kidney of mice treated with epicarp of 45 mg/kg body weight and endo mesocarp of 75 mg/kg body weight.

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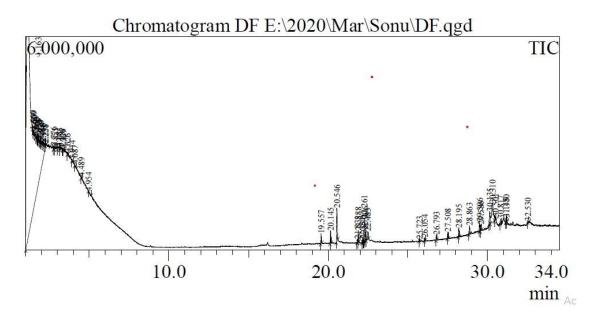
Keywords:-Diabetes, Alloxan, Selencereus undatus, Epicarp, Endomesocarp.

#### **INTRODUCTION:-**

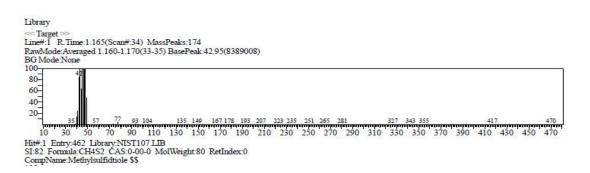
Selenicereus undatus commonly called pitaya or dragon fruit, belongs to Cactaceae family and is widely cultivated in Mexico, Southeast Asia, India, The,U. S. A, Australia, and throughout Tropical and Sub tropical regions. S. undatus is characterized by a pink epicarp and white-fleshed pulp containing edible black seeds in its endocarp. The fruit is oblong to oval, measuring 6-12cm in length, 4-9 cm thickness, with large bracteoles. S. undatus contains bioactive compounds such as polyphenols, vitamin C and major antioxidants pigments, including betacynains and betaxanthins. Over recent years, it has attracted significant research interest due to its phytochemical constituents, which exhibit tremendous pharmacological properties. Studies suggest that S. undatus extract may play a vital role in the treatment and prevention of various diseases. One study found that dragon fruit effectively reduces aortic stiffness, particularly in diabetic subjects. Regular consumption of dragon fruit has been shown to lower the risk of thrombosis and slow the plaque formation, thereby reduces coronary inflammation. Oxidative reactions generate free radicals which contribute to chronic diseases. Fortunately, S. undatus has antioxidants such as flavonoids, terpenoids, tannins, betalains and many more its

derivatives to minimize the catalytic escalation of bad cholesterol and tumor. Additionally, pectin in dragon fruit consists of galacturonic acid, which can clear clogged blood vessels and maintain optimal blood pressure. The lycopene present in S. *undatus*, has has demonstrated protective effects against prostate cancer and breast cancer.

TABLE:-1 GCMS REPORT OF SELENINCEREUS UNDATUS FRUIT EXTRACT

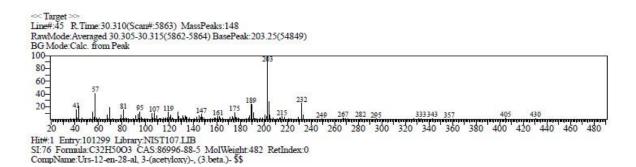


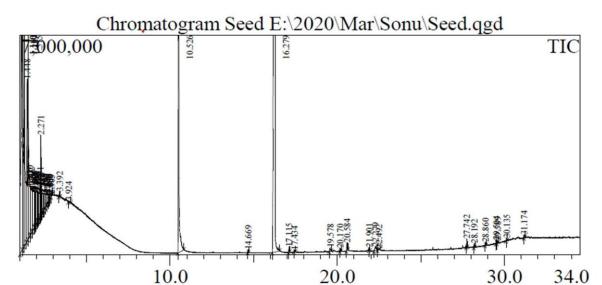
Name	Name
Methylsulfidtiole	1-Propyne, 3,3'-[ethylidenebis(o:
Cyclobutanol	1,2-Propadiene
1-Propyne	2-Butynoic acid
Ethanol, 2-(2-propynyloxy)-	2,5-Furandione, dihydro-3-methy
3-Butyn-1-ol	Argon Argon
3-Butyn-1-ol	1-Propyne
Oxirane, ethenyl-	Silane, diethoxydimethyl-
9-Octadecenoic acid, (E)-	Argon
Octadecanoic acid	3-Butyn-1-ol
Phthalic acid, diisooctyl ester	Argon
Pentadecane	Argon
Nonadecane	Argon
Tetratriacontane	1,2-Propadiene
Heptacosane	2,5-Furandione, 3-methyl-
Tetratriacontane	2-Butynoic acid
Heptacosane	Argon
.gammaSitosterol	3-Butyn-1-ol
Tetratriacontane	1,2-Benzenedicarboxylic acid, b
	Hexadecanoic acid, methyl ester
Urs-12-en-28-al, 3-(acetyloxy)-,	n-Hexadecanoic acid
Urs-12-en-3-ol, acetate, (3.beta.)	11.14-Eicosadienoic acid, methy
Heptadecane, 2,6,10,15-tetramet	10-Octadecenoic acid, methyl es
7,7-Dimethoxy-2,3,4,5,6,7-hexal	Octadecanoic acid, methyl ester
Guanosine, 2'-O-methyl-	1.E-11.Z-13-Octadecatriene
2-Methyl-6-(5-methyl-2-thiazolii	9-Hexadecenoic acid



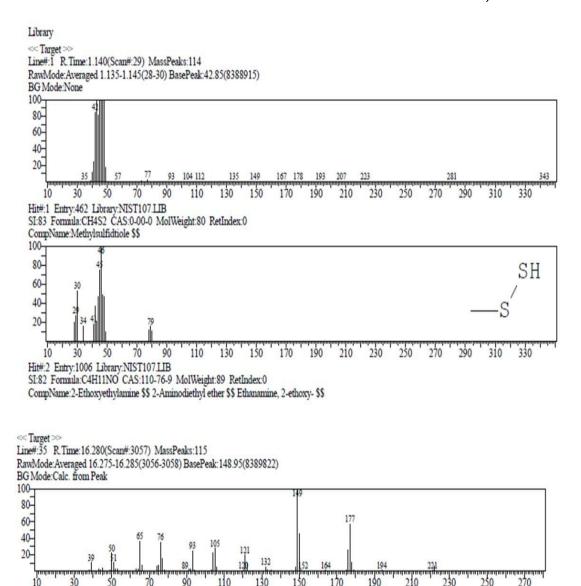
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# TABLE-2 GCMS REPORT SELENINCEREUS UNDATUS SEED EXTRACT





Name	Name
	Argon
Methylsulfidtiole	1,2-Propadiene
Isopropyl Alcohol	Argon
1-Propene, 2-fluoro-	3-Butyn-1-ol 2-Butynoic acid
Ethyl Acetate	3-Butyn-1-ol
	Propanoic acid, ethyl ester
1-Propanol, 2-methyl-	3-Butyn-1-ol
2-Butenal	Argon
Ethane, 1-ethoxy-1-methoxy-	Argon
	1,3-Dioxolane, 2,4,5-trimethyl-
1,2-Benzenedicarboxylic acid, b	Ethane, 1,1-diethoxy-
Octadecanoic acid, methyl ester	Argon 1,2,5-Oxadiazole
1,2-Benzenedicarboxylic acid, b	1-Propyne
10-Octadecenoic acid, methyl es	Argon
E-7-Tetradecenol	Argon
	3-Butyn-1-ol
Tetradecanoic acid	Cyclopropene
Farnesol isomer a	Argon 3-Butyn-1-ol
Nonadecane	3-Butyn-1-ol
Tetratriacontane	Boron, carbonyltrihydro-, (T-4)-
	Butanoic acid, ethyl ester
Tetratetracontane	Argon
.gammaSitosterol	Methyl Salicylate
Heptacosane	3-Isopropoxy-1,1,1,7,7,7-hexam Phthalic acid, di-(1-hexen-5-yl)
3.alpha(Trimethylsiloxy)choles	N-(Trifluoroacetyl)-N,O,O',O"-te
timpin (timitaly) blicks	Benzoic acid, 2-(1-oxopropyl)-



#### **OBJECTIVES:**

Hit#:1 Entry:79730 Library:NIST107.LIB

CompName:Phthalic acid, di-(1-hexen-5-yl) ester \$\$

SI:84 Formula:C20H26O4 CAS:0-00-0 MolWeight:330 RetIndex:0

In order to justify the hypothesis, the following well defined objectives are:- 1. Identification of the plant for taxonamical position with the help of botanist.

- 2. Crude Extraction and Isolation of extract from dragon fruit(epicarp and endocarp) by performing solvent extraction method(Ridge National Laboratory in 1950).
- 3. To study the antidiabetic activity of crude extract administered in diabetic mice with the following:-
- a.) Hametological parameters Blood glucose, Glycosylated glucose, Triglycerides, Cholesterol.
- b.)Liver function test transaminase (SGOT,SGPT).
- c.)Renal function test Serum creatinine and urea.
- d.)Histology-Liver, Kidney and Pancreas.

#### MATERIALS AND METHODOLOGY

• Collection of the fruit - Fresh fruits of *Selenicereus undatus* were purchased from Deccan exotics farm, sangareddy, Telangana. The fruits were washed thoroughly with water and kept for air dry. The fruit peel, pulp, seed were separately grounded in an electric mixer and used for further use.

- Preparation of extract- Solvent extraction method by using soxhelation. The dried powdered of 500gms of each is kept for sohxelation in 1500ml of ethanol solvent and runned around for 3-8 cycles at boiling point  $70^\circ$  c. The ethanolic extract was concentrated using a rotary evaporator at room temperature 37 c. The extracted crude is collected in petridish, kept for air dry. Then the ethanolic extract was stored in an air tight container for future use.
- Experimental Animals –Swiss Albino male mice weighing about 25-30 g were obtained from the animal house of Vyas Labs, Medchal, Malkajigiri District, Telangana, and used for the study. Mice were housed at constant room temperature of 37°C with a 12-hour light, 12-hour dark cycle, and fed on pellets with free access to tap water. The work has been taken with due concern of approval by the Ethical Committee. All the experiments were carried out according to the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.
- Preparation of the test sample Alloxan monohydrate 150mg/kg body weight IP is used to induce diabetes in healthy mice. To treat alloxan induced diabetic mice the standard drug metformin (75mg/kg body weight) is been administered.
- Acute toxicity study It is to be carried out to determine the dose of fruit extract (epicarp and endocarp) given to overnight fasted healthy mice.
- Experimental design Albino Mice were used for the experimentation. Housing and acclimatization is to be done in animal house on facility of the department.
- After acclimatization the animals were randomized into different groups as group I (control- non induced and not administered the fruit extract); group II (induced mice received Alloxan); group III (induced mice receives metformin); group IV A,IV
   C(induced mice epicarp extract with 45 mg/kg body weight); group IV B,IV D (induced mice-endocarp extract with 75mg/kg body weight); group V(non induced and administered with whole fruit extract), followed by biochemical analysis.

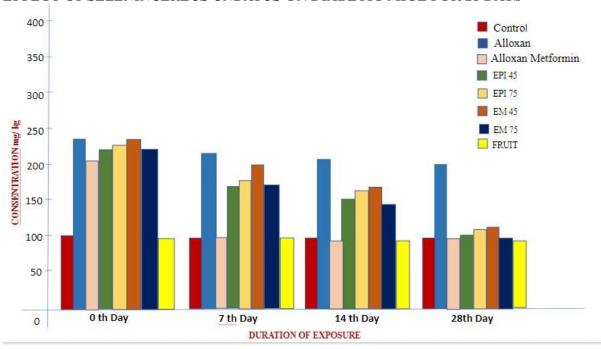
### **PROTOCOLS:**

- Haematological evaluation Haemoglobin estimated by Sahli's method(1894).
- Blood Glucose:- Estimated by Nelson and somogayi method(1957).
- Glycosylated glucose by Bookchin and Gallop et al .,(1969)
- Serum Glutamic Oxaloacetic Transaminase(SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) were measured by the method of Reitman and Frankel et al., (1957).
- Kidney Function test: Serum creatinine level estimated by **Jaffe** *et al.*,(1967).
- CHOLESTEROL TEST:-GPO-POD by Liebermann Burchard
- TRIGLYCERIDES TEST:-CHO-POD by Kesseler and Lederer

## STATISTICAL ANALYSIS:

- The results tested for significance using descriptive statistics, analysis of variance(ANOVA) for comparision of control and experimental groups among mean values and paired t-test for comparison of mean scores of pre and post values among control and experimental groups.
- POST-Hoc test also performed at % level of significance and are depicted in the form of bar charts, multiple bar charts which equivalently represent the data of Mean ±Standard deviation, standard error of the Mean.
- Statistical analysis were performed using SPSS version 26.0 software.





# AVERAGES OF 0TH DAY,7TH DAY,14TH DAY,28TH DAY OF BLOOD GLUCOSE

Table-3								
PARAMETE RS	CONTROL	ALLOXAN	ALN+M	ALN+EP45	ALN+EP75	ALN+EM45	ALN+EM75	FRUIT
0 th day	98.83±2.63	246.5±9.09	140.50 ±8.06	233.17 ±8.88	233.50±8.78	237.83 ±8.81	231.83 ±8.72	96.67±4.3
7th day	99.67±3.93	239.17 ±8.75	100.00 ±5.25	183.50 ±6.15	190.17 ±14.90	200.50 ±11.11	179.50 ±6.89	100.50±4.9
14 th day	100.00±4.60	7,1,1,1	98.83 ± 1.72	165.33 ±6.43	172.17 ± 10.68		161.50 ±3.78	99.33±3.7
28th day	239.67 99.50±3.93±5.21		100.83 ±4.26	111.83 ±7.88.	122.33 121.00 ±2.96±3.77.		107.66 ±8.33	98.33±2.1

- The above result represent the Mean  $\pm$  Sd values with multiple bar diagram.
- ALN:- When we compare alloxan induced mice at day wise ,the initial day of mean value of blood glucose was  $246.50 \pm 9.09$  were on 7 th day it has decreased with a mean value of  $239.17 \pm 8.75$ . again on  $14^{th}$  day the mean value was  $241.17 \pm 6.85$  and on 28 th day it was  $239.67 \pm 5.24$ .
- ALN+M:- When we compare alloxan + metformin treated mice at day wise ,the initial day of blood glucose was  $140.50\pm8.06$  were on 7 th day it has decreased with a mean value of  $100.00\pm5.25$  again on  $14^{th}$  day the mean value was  $98.83\pm1.72$  and on 28 th day it was  $100.83\pm4.26$ .
- ALN+EP45:- When we compare alloxan + EP45 treated mice at day wise ,the initial day of blood glucose was  $233.17 \pm 8.88$  were on 7 th day it has decreased with a mean value of  $183.50 \pm 6.15$  .again on  $14^{th}$  day the mean value was  $165.33 \pm 6.43$  and on 28 th day it was  $111.83 \pm 7.88$ .
- ALN+EP75:- When we compare alloxan + metformin treated mice at day wise ,the initial day of blood glucose was  $233.50\pm8.78$  were on 7 th day it has decreased with a mean value of  $190.17\pm14.90$  again on  $14^{th}$  day the mean value was  $172.17\pm10.68$ and on 28 th day it was  $121.00\pm2.96$ .
- ALN+EM45:- When we compare alloxan + metformin treated mice at day wise ,the initial day of blood glucose was 237.83  $\pm 8.81$  were on 7 th day it has decreased with a mean value of  $200.50 \pm 11.11$  again on  $14^{th}$  day the mean value was  $176.50 \pm 8.55$  and on 28 th day it was  $122.33 \pm 3.77$ .

• ALN+EM75:- When we compare alloxan + metformin treated mice at day wise ,the initial day of blood glucose was 231.83 ±8.72 were on 7 th day it has decreased with a mean value of 179.50 ±6.89 again on 14<sup>th</sup> day the mean value was  $161.50 \pm 3.78$  and on 28 th day it was  $107.66 \pm 8.33$ .

## **HISTOLOGY:**

The investigation of kidney ,liver, pancreas treated with crude extract of S.undatus.

# KIDNEY Figure no:- 1-100X magnification. Figure no :- 2- 100X magnification. Figure no:-3- 100X magnification 1 CN- Normal morphology of tubules -ALN-Alloxan induced mice shows ALN+M -Normal morphology of glomerulus green arrow moderate tubular and interstitial in cortex region - red arrow Normal morphology of glomerulus -red inflammation -green arrow with infiltration of inflammatory cells in Normal morphology of tubules - green glomerulus- red arrow Figure no:-5- 100X magnification Figure no:-3 100X magnification. Figure no:- 6- 100X magnification Figure no:- 4- 100X magnification ALN+EP75-Normal morphology of ALN+M -Normal morphology of ALN+EM45-Normal morphology of ALN+EP45-Normal morphology of glomerulus and no pathological glomerulus in cortex region - red tubules - green arrow tubules and no inflammation was changes were observed -red arrow Normal morphology of glomerulus - red observed - Green arrow Normal morphology of glomerulus Normal morphology of tubules -Normal morphology of glomerulus and no pathological changes were and no pathological changes were Normal morphology kidney and no green arow observed -red arrow abnormality were observed observed [red arrow]

Figure no:-3- 100X magnification ALN+M -Normal morphology of glomerulus in cortex region - red arrow

Normal morphology of tubules - green

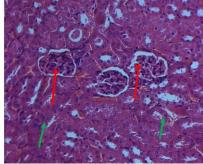


Figure no:- 7 -100X magnification ALN+EM75-Normal morphology of glomerulus - red arrow Normal morphology kidney and no abnormality were observed Normal morphology of tubules - green arrow

Figure no:- 8- 100X magnification FRT-Normal morphology kidney and no abnormality were observed in glomerulus and tubulus

# **PANCREAS**

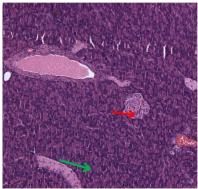


Figure no:- 1- 100X magnification -CNT- No abnormality detected Glandular pancreas: beta cells appeared normal

in islets of langherhans -red arrow

Acinar cells in non glandular region appeared normal - green arrow

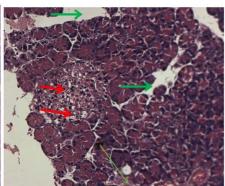
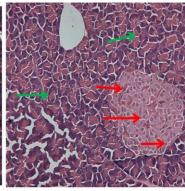


Figure no:- 2- 100X magnification ALN-Moderate degeneration and apoptosis of beta cells were observed - red arrow Peri ductular and peri vascular fibrosis- green



Normal morphology of acinar cells - green arrow

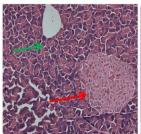


Figure no:- 3- 100X magnification ALN+M-Normal morphology of beta cells in islets of pancreas – red arrow Acinar cells appeared normal – green arrow

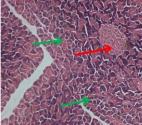


Figure no:-4- 100X magnification ALN+EP45-Normal morphology of acinar cells – green arrow Normal morphology of pancreas and could not find any variation

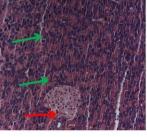


Figure no:-5- 100X magnification ALN+EP75-Normal acinar cells in pancreas – green arrow

Normal morphology of beta cells in islets of pancreas with no hemorrhages – red arrow

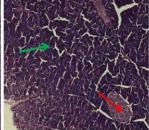


Figure no:-6 -100X magnification ALN+EM45-Glandular cells of pancreas – islets of pancreas appeared normal -red arrow

Acinar cells appeared normal – green arrow

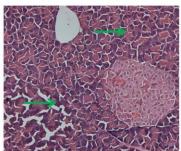


Figure no:- 3 -100X magnification ALN+M-Normal morphology of bet cells in islets of pancreas – red arrow Acinar cells appeared normal – green arrow

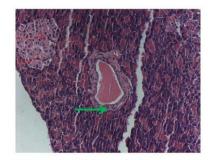


Figure no:-7- 100X magnification ALN+EM75-Beta cells are appeared normal in islets of pancreas – Red arrow Ductular pancreas appeared normal with secretions – green arrow

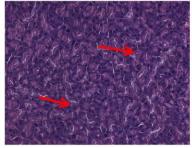


Figure no:- 8 -100X magnification FRT-Acinar cells in non glandular region appeared normal - arrow

#### LIVER



Figure no:-I -100X magnification CNT-Normal morphology of portal vein , portal region with bile duct and hepatic artery – green arrow Normal morphology of hepatocytes were observed – red arrow

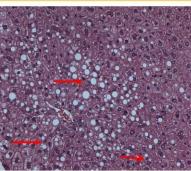


Figure no:- 2 -100X magnification ALN-Severe Macro vesicular vacuolar / fatty degeneration noticed in centri lobular region of liver Mild foci of necrosis and fibrosis noticed in centri lobular region of liver

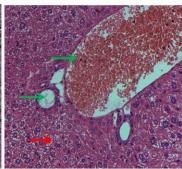


Figure no:- 3 -100X magnification ALN+M-Normal morphology of hepatocytes were observed Normal morphology of bile duct – red arrow Normal morphology of liver and no abnormality were observed

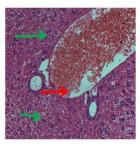


Figure no:- 3- 100X magnification ALN+M-Normal morphology of hepatocytes were observed Normal morphology of bile duct – red arrow

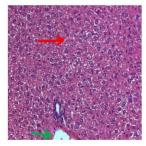


Figure no:- 4-100X magnification ALN+EP45-Normal morphology of portal vein



Figure no:- 5 -100X magnification ALN+EP75-Normal morphology of liver and no abnormality were observed

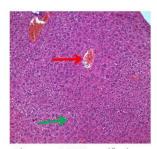


Figure no:- 6 -100X magnification ALN+EM45-Normal morphology of hepatocytes were observed

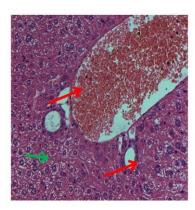


Figure no:- 3- 100X magnification ALN+M-Normal morphology of hepatocytes were observed Normal morphology of bile duct – red arrow

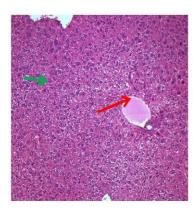
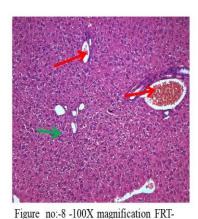


Figure no:-7- 100X magnification ALN+EM75-Normal morphology of portal vein , portal region with bile duct and hepatic artery –red arrow



Normal morphology of hepatocytes were observed
Normal morphology of portal vein , portal region with bile duct and hepatic artery

**RESULT:** The oral administration of the ethanolic extract of *Selenicereus undatus* reduces blood glucose and serum lipid levels, which may be due to improved insulin secretion through the recovery of pancreatic cells. The presence of alkaloids, flavonoids, linolenic acid, phthalic acid, arginine, and propanoic acid in the crude extract of *S. undatus*. Epicarp extract (45 mg/kg body weight) and endomesocarp extract (75 mg/kg body weight) shown more efficacy than the other experimental groups .It has demonstrated its beneficial role in diabetes management, as evidenced from this study.

Therefore, the findings suggest that this crude extract could serve as an alternative complementary therapeutic supplement, potentially replacing synthetic drugs. However, further studies are required to establish the exact mechanism of action, targeting the specific lead molecule responsible for its antidiabetic effects.

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