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Investigation Of Antidiabetic Activity Of Artrocarpus Altilis In Alloxan Induced Diabetic Rats

Akanksha Singh[1*], Anubhav Dubey[2], Shyam Singh[1], Bhupendra Patel[2], Alok Kumar Shukla[3]

[1] Research Scholar, Department of Pharmacology, Advance Institute of Biotech and Paramedical Sciences Kanpur Uttar Pradesh India.

[2]Assistant Professor, Department of Pharmacology, Maharana Pratap College of Pharmacy Kanpur (U.P.) – India.

[3]Assistant Professor, Department of Basic Science and Humanities, Maharana Pratap Group of institution

(U.P.) – India.

Email- yadavak.akanshasingh17@gmail.com

ABSTRACT

An overview of the Antidiabetic activity of Atrocarpusaltilis seeds on alloxan induced diabetic rats is presented in this research work. The study aimed at evaluating antidiabetic activity of the ethanolicseed extract of A. altilisin alloxan-induced diabetic rats. Diabetes was induced in rats after overnight fast with 120 mg/kg alloxanintraperitoneally. After 72 h, those with plasma glucose levels >200 mg/dl were classified as diabetic. Six diabetic rats in each group were treated daily for 14 days orally with 250, and 350 mg/kg of the extract, glibenclamide(10 mg/kg), while another group was untreated. Control received 5 ml/kg of 0.9% saline solution.. Effects of extract on glucose, and body weight were evaluated. Percentage inhibition was determined.Diabetic control was achieved on the 7th day of the study with 250, and 350 mg/kg of the extract showing glucosereduction of 69.23% and 75.21%, respectively. This study showed that seeds ofA. altilishave antidiabetic activity.

Keywords- Glibenclamide Atrocarpusaltilis, Alloxan, Diabetes.

INTRODUCTION

Diabetes mellitus (DM) is defined as a disease or chronic metabolic disorder with multiple etiologies is characterized by high blood glucose levels with impaired metabolism of carbohydrates, lipids and proteins as a result of insufficiency of insulin function. (Brownlee M 2001) Insulin is ahormone manufactured by the beta cells of the pancreas, which is required to utilize glucose from digested food as an energy source. (Babenkovaet al. 1999) Chronic hyperglycemia is associated with microvascular and macrovascular complications that can lead to visual impairment, blindness, kidney disease, nerve damage, amputations, heart disease, and stroke. In 1997 an estimated 4.5% of the US population had Diabetes.(NDA 2011).

Breadfruit (Artocarpusaltilis) is one of the most significant trees in tropical homegardens and perhaps the most widespread and useful tree in the important genus Artocarpus. It is a medium-size evergreen tree typically reaching 8–25 m (26–82 ft) in height that is easily recognized by its fruit, the largest among cultivated plants. The succulent, aromatic, and flavorful fruit is eaten fresh or preserved in myriad ways. (Umesh JB et al. 2010) The leaves and fruit waste provide valuable fodder for cattle, pigs, and goats. Many parts of the plant including the bark, roots, leaves, and fruit are attributed with medicinal properties. Wood chips yield a dye used to give the famous orange-red color to the robes of Buddhist priests. The tree can provide many environmental services. (Soobratteeet al. 2005) It is highly wind tolerant and therefore makes a good component in a windbreak or border planting. Growing in pastures, it can provide fallen fruit for livestock, shade, and long-term timber. In homegardens, the dense breadfruit canopy can provide a visual screen and is very ornamental. Introduced to most Pacific islands after European contact, the tree can be found throughout the Pacific, mainly in homegardens, where it finds a place among other favorite multipurpose plants. It is easy to grow and more adaptable than some of the other common Artocarpus species such as breadfruit (A. altilis). It is not considered to be an invasive species. (Samaddar HM et al. 1985)

Fruit is rich in dietary fibre, which makes it a good bulk laxative. The fibre content helps protect the colon mucous membrane by binding to and eliminating cancer-causing chemicals from the colon.Fresh fruit has small but significant amounts of vitamin-A, and flavonoid pigments such as carotene-ß, xanthin, lutein and cryptoxanthin-ß. Together, these compounds play vital roles in antioxidant and vision functions. Vitamin A is also required for maintaining integrity of mucusa and skin. Consumption of natural fruits rich in vitamin-A, and carotenes has been found to protect from lung and oral cavity cancers.Jackfruit is a good source of antioxidant vitamin-C, provides about 13.7 mg or 23% of RDA. Consumption of foods rich in vitamin C helps the body develop resistance against infectious agents and scavenge harmful free radicals.It is one of the rare fruits that is rich in B- complex group of vitamins. It contains very good amounts of vitamin B-6 (pyridoxine), niacin, riboflavin, and folic acid.Further, fresh fruit is a good source of potassium, magnesium, manganese, and iron. Potassium is an important component of cell and body fluids that helps controlling heart rate and blood pressure.

MATERIALS AND METHODS

Experimental Rodents

Wistar albino rodents of either sex weighing between 150-200g were used for this study. They were procured in the AIBPS animal house Kanpur recognized by the Institutional Animal Ethics Committee (IAEC). Polypropylene limits were used to house (3 for each pen) the animal at a temperature of 28 ±50C and 12 h light /dullcycle. Hindustan Lever chow pellets were used to feed the animal and water not basic. The animals were kept fasting medium –term going before the examination and this study was approved by IAEC for animal studies (1122/PO/Re/S/2020/CPCSEA) include all framework used in the research.

Drugs and Chemicals

Ethanol, Chloroform, Sodium Hydroxide, Hydrochloric Acid Benzene, Magnesium were obtained from Himedia Laboratories, Mumbai , Nitric Acid Glaxo Smith Kline Pharmaceutical Ltd. , Mumbai and Alloxan was purchased from Sigma-Aldrich.

Collection and authentification of plant

The seeds of Artocarpusaltiliswere collected locally in the month of July from market. Herbarium file of plant part was prepared & authenticated by the botanist and. The voucher specimen was deposited MePl/SBRL/121.

Drying and size reduction of plant material

The seeds of Artocarpusaltiliswere dried under shade in laboratory. They were pulverized to make coarse powder. The coarse powder of seeds was passed through sieve No.18 to it's maintain uniformity and stored in cool and dry place for further study.

Screening of Powder (Physiochemical and Phytochemical Analysis)

Physiochemical screening of powdered seeds was done by the standard reported methods.

Extraction of seeds of Artocarpusaltilis

Extraction of seeds of Artocarpusaltiliswas done by Soxhlet extraction method.

Soxhlet Extraction

Soxhlet apparatus was used for the solvent extraction and ethanol was selected as a solvent for extraction while petroleum ether was used for defatting of the waxy materials. The method is continuous hot percolation method in which the selected solvent is vaporized and is continuously passed through the drug material kept in the percolator. The movement of fresh solvent through the powdered drug material extract out the constituents in the solvent. The non polar solvents remove the non polar constituents from the crude drug matrix e.g. fats and lipids. The petroleum ether helps in the process.

Preparation of extract formulation

A suspension formulation of ethanolic extract of Atrocarpusaltilisin 0.5% Carboxy Methyl Cellulose solution was prepared for further in-vivo pharmacological study. The suspension formulation was prepared for the ease to administer into the animals.

Induction of type 1 diabetes (alloxan model)

Alloxan monohydrate was dissolved in saline and administered intraperitoneally into fasted rats at a dose of 120 mg/kg body wt. The solution should be fresh and prepared just prior to the administration. The rats were given 5% (w/v) glucose solution in feeding bottles for next 24 h in their cages to prevent hypoglycaemia after alloxan injection. After 72 h rats with blood glucose level (BGL) greater than 200 mg/dl and less than 400 mg/dl were

selected andobserved for consistent hyperglycaemia (fasting blood glucose level greater than 200 mg/dl and lesser then 400 mg/dl) upto 7 days. The treatment was continued for the next 14 days and blood samples were collected. During the treatment period, the weight of the animals and the fasting blood glucose (FBG) measurements were determined with a weighing scale and a glucometer (using the tail vein), respectively, every 2 days from the beginning of the treatment (day 1) to the last day of the experiment (15th day). The percentage change of body weight was calculated from its initial weight. Alloxan may cause severe ketoacidosis and may lead to death of animal. In view of this the mortality rate was monitored throughout the study.

Experimental Protocol (Anti Diabetic)

In this experiment, the rats were divided into different groups as per defined protocol- Group I: Normal Control Group (0.9% saline; 5 mL/kg body weight orally for 14 days.

Group II: Diabetic Group (Alloxan monohydrate i.p.120 mg/kg) in addition with 5% (w/v) glucose solution in feeding bottles for next 24 hrs.

Group III: Standard Treated Group Glibenclamide (10 mg/kg, p.o.) for 14 days. Group IV: Treated with defined dose of test drug 250mg/kg (p.o.) for 14 days. Group V: Treated with defined dose of test drug 350mg/kg (p.o.) for 14 days.

Evaluation Parameters:

A) Blood Glucose level measurement – 0th day, 1, 3, 5, 7, 9, 11, 13 and 15th day.

B) The deviation of body weight of the animals- 0th day, 1, 3, 5, 7, 9, 11, 13 and 15th day.

Acute Toxicity Studies

All aqueous-treated rats showed no discernible behavioral changes up to 2000 mg/kg by oral route. No mortality was observed at this dose during 72 h observation period and no significant body weight changes were observed during 14 days toxicity study.

Statistical analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.). The results are analyzed for statistical significance using one-way ANOVA followed by Dunnett'stest.P< 0.05 was considered significant.

RESULT

Table No.1:- Morphological characteristics of Artrocarpusaltilisseeds

S.No.	Character	Observation

1	Color	Yellowish
2	Odour	None
3	Taste	Acrid
4	Size	4.5-7.5 cm in length
5	Texture	Rough

Table No. 2:- Physiochemical analysis of powder of Artrocarpusaltilisseeds

S.No.	Parameters	Observation (% W/W)
1	Loss on drying	0.51
2	Total ash value	6.22
3	Acid insoluble ash value	3.95
4	Water soluble ash value	1.17
5	Foaming index	6.9 (ml)

Table No. 3:- Consistency and color of T Artrocarpusaltilisseeds extract

Extract	Color	Consistency	Percentage Yield (%W/W)
Ethanolic	Brownish green	Semisolid	10. 26 %

Table No. 4:- Phytochemical screening of ethanolicextract of Artrocarpusaltilis seeds

S.No.	Chemical Tests	Ethanolic extract

1	**Carbohydrates** i) Molisch'sTest ii) Fehling'sTest iii) Benedict'stest	 (+) (+) (+)
2	**Tannins** i) with 5%ferric chloride solution ii) with 10% aqueous Potassium dichromate solution iii) with 10% lead acetate solution	 (+) (-) (-)
3	**Alkaloids** i) Dragendorff'sTest ii) Mayer'sTest iii) Hager'sTest	 (+) (+) (+)
4	**Glycosides** i) Borntrager'sTest ii) LegalTest iii) BaljetTest	 (+) (+) (+)

5	**Flavonoids** i) Shinoda'sTest ii) Alkaline reagenttest iii) Leadtest	(+) (+) (+)
6	**Steroids and Sterols** (I)Libermann-BurchardTest II) Salkowski Test	(–) (–)
7	**Proteins and Amino Acids** (i) BiuretTest (ii) NinhydrinTest (iii) Millon's Test	(+) (-) (-)

Table-5 Effect of the ethanolic extract of A. altilisseeds (EAA) on blood glucose (mg/dl) in alloxan- induced diabetes mellitus

S.No	Days	Treatment Groups				
		G-I Control	G-II Diabetic Group	G-III Standard	G-IV Treatment 1st (250)	G-V Treatment 2nd (350)
1	0	87.15±5.05	90.44±6.59	83.35±4.65	73.51±5.42	75.67±10.52
2	1	86.77±9.86	288.36±11.40*	336.21±11.46*	331.71±10.23*	321.09±13.03*
3	3	83.35±4.44	294.51±5.98*	224.58±5.04*	271.04±4.81*	231.53±4.95*
4	5	87.48±10.19	303.42±11.73*	186.5±11.79	118.9±10.56	98.08±11.63
5	7	89.34±12.25	307.17±13.79*	140.61±10.85	102.08±12.62	79.61±5.47

6	9	86.55±5.83	317.82±7.37*	131.49±5.43	95.81±6.20	76.66±13.45
7	11	90.14±10.64	324.5±12.18*	123.17±12.24	100.55±11.01	79.08±14.37
8	13	87.52±5.22	333.89±6.76*	114.56±5.82	92.83±5.59	73.52±7.54
9	15	88.47±10.97	339.18±12.51*	105.78±12.57	89.56±11.34	72.71±2.59

All values are mean ± SEM, n=6. *Significant difference ($p<0.05$), test groups vs. control

Fig. 1 Effect of EAA on Blood Glucose Level in Rats on Different Days

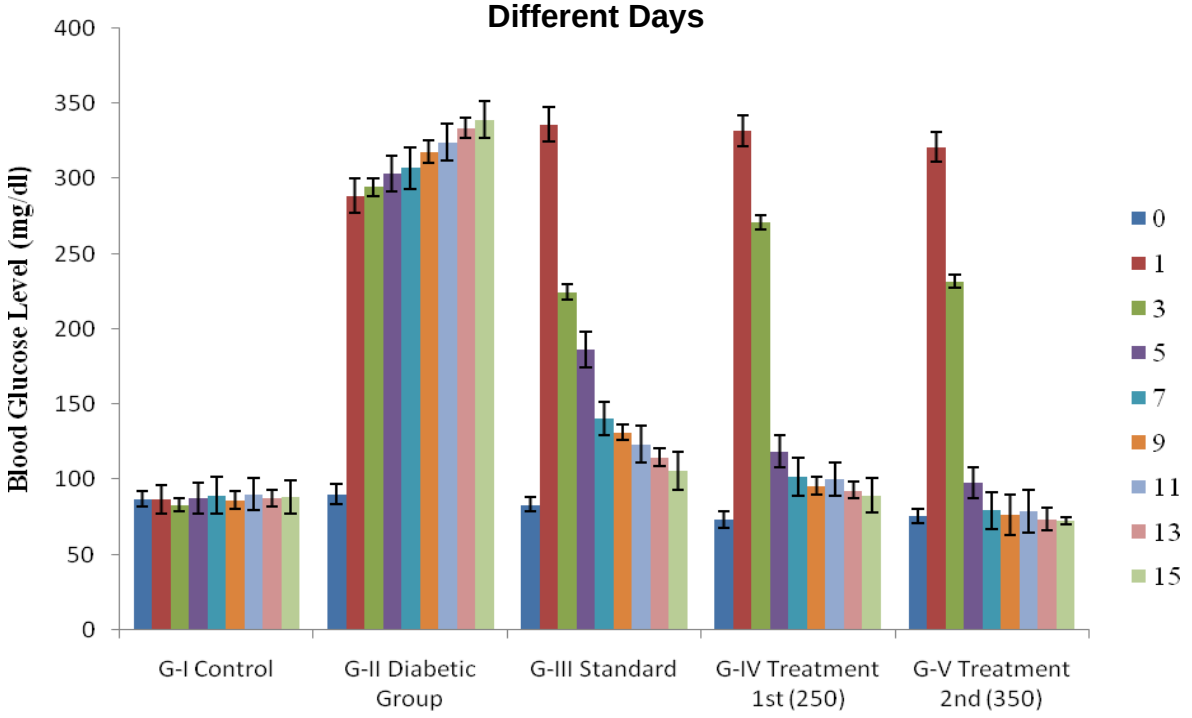


Table-6 Percent Reduction in Blood Glucose Level (mg/dl) in Rats

S. No.	Days	Treatment Groups				
		G-I Control	G-II Diabetic Group	G-III Standard	G-IV Treatment 1st (250)	G-V Treatment 2nd (350)
1	**0**	0	0	0	0	0
2	**1**	0	0	0	0	0
3	**3**	0	-2.13	33.20	18.29	27.89
4	**5**	0	-5.22	44.53	64.16	69.45

5	**7**	0	-6.52	58.18	69.23	75.21
6	**9**	0	-10.22	60.89	71.12	76.13
7	**11**	0	-12.53	63.37	69.69	75.37
8	**13**	0	-15.79	65.93	72.01	77.10
9	**15**	0	-17.62	68.54	73.00	77.36

Fig. 2 Percentage Reduction in Blood Glucose Levels in Rats

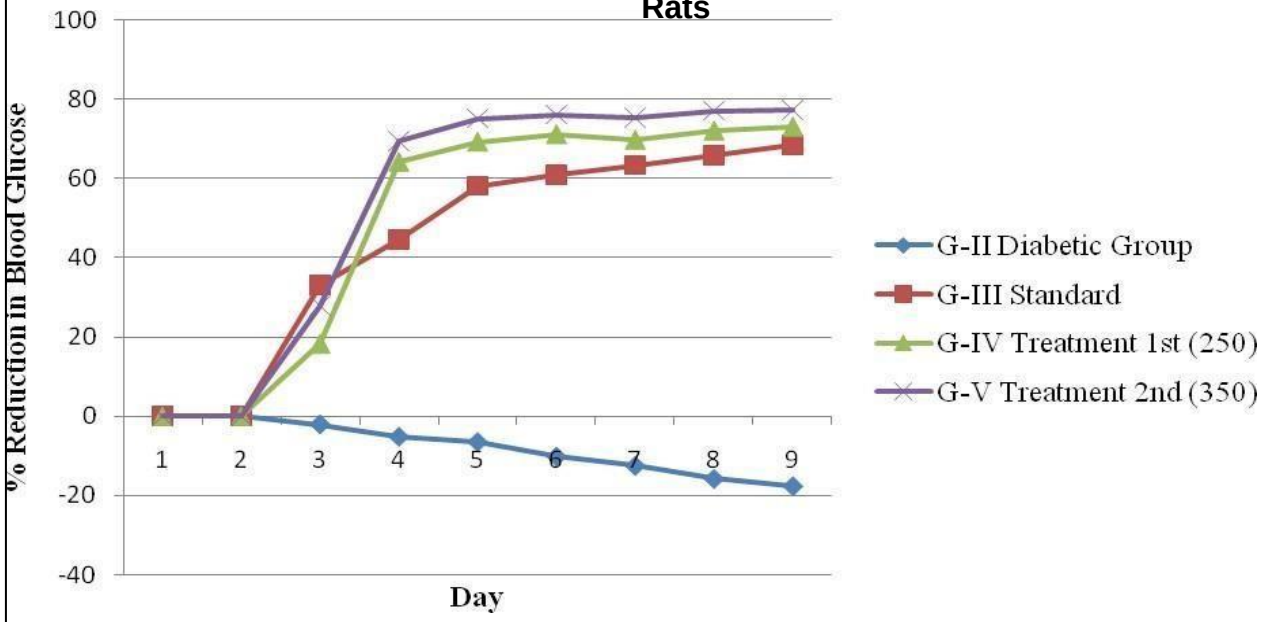
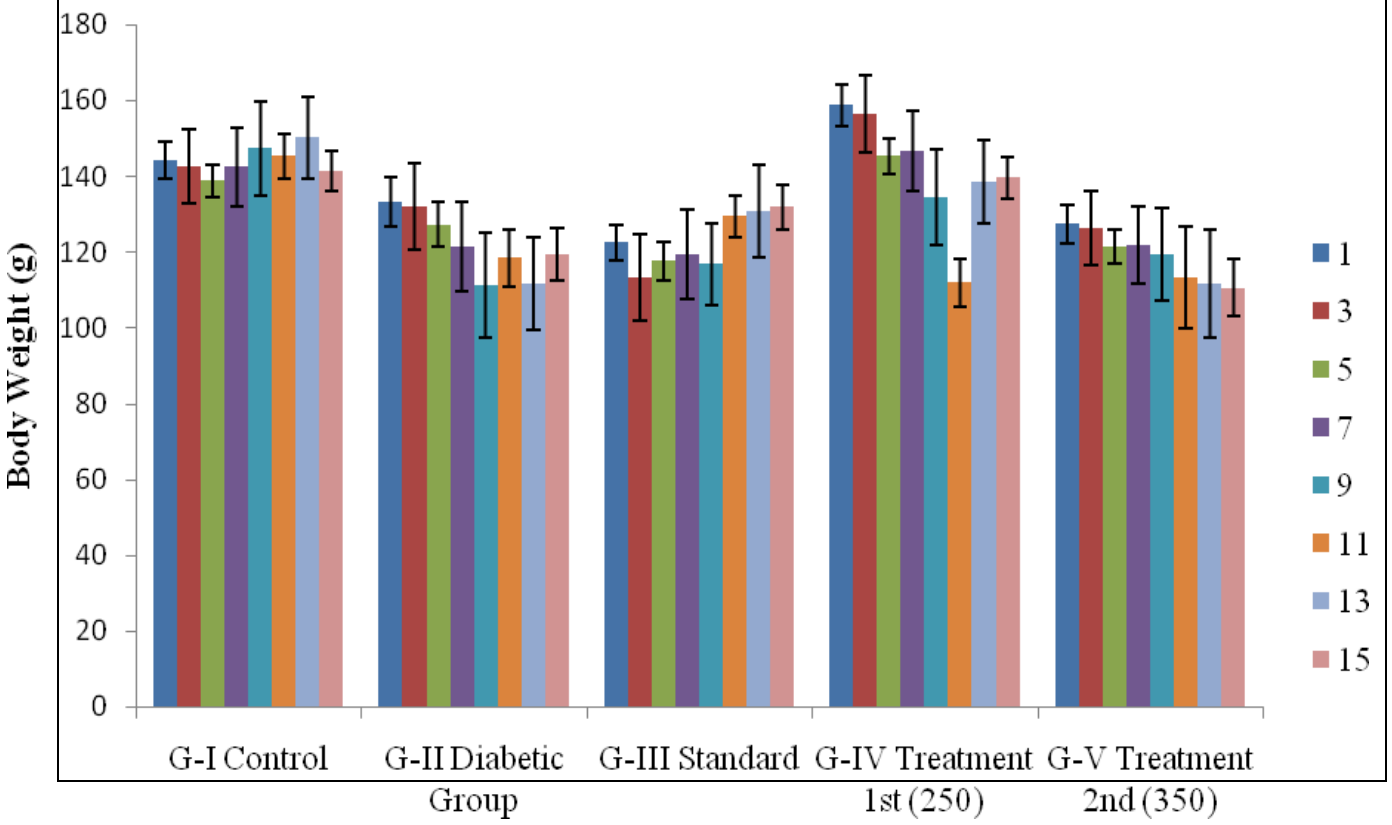


Table-7.Effect of the EAA on Body Weight (in g)

S. No.	**Days**	**Treatment Groups**				
		G-I Control	**G-II Diabetic Group**	**G-III Standard**	**G-IV Treatment 1st (250)**	**G-V Treatment 2nd (350)**
1	**1**	144.2±4.94	133.2±6.24	122.4±9.49	158.7±5.99	127.4±6.26
2	**3**	142.5±1.48	132.1±0.82	113.2±3.07*	156.4±2.57	126.4±3.18
3	**5**	138.7±5.27	127.2±6.57	117.6±9.82*	145.3±6.32	121.3±6.86*
4	**7**	142.4±7.33	121.3±8.63	119.5±8.88*	146.7±8.38	121.7±4.70*
5	**9**	147.3±2.91	111.2±2.21	116.8±3.46*	134.5±1.96	119.5±5.68*
6	**11**	145.2±5.72	118.4±7.02	129.4±10.27	111.8±6.77*	113.4±6.60*

7	**13**	150.1±2.30	111.5±1.60	130.7±3.85*	138.5±1.35	111.6±2.77*
8	**15**	141.4±6.05	119.4±7.35	131.9±10.60*	139.6±7.10	110.5±2.18*

Fig. 3 Effect of EAA on Body Weight



All values are mean ± SEM, n=6. *Significant difference (p<0.05), test groups vs. control

DISCUSSION

A. altilis has been used traditionally in folk medicine to treat several diseases. The present study was designed to evaluate the potential and mechanism of anti diabetic activity of A. altilisethanolic extract in alloxanindiuced diabeticrats.After the extraction, pharmacognostical evaluation was done including determination of Ash value in which percentage of Water soluble ash, Acid insoluble ash, Total ash and moisture content, foaming index were determined. Extract was subjected to various chemical tests for preliminary identification of various phytoconstituents. The extract contains carbohydrates, alkaloids, flavonoids, glycosides (Mukherjee 2008 and Samaddar HM 1985).

This study was undertaken; mainly to assess the protective effect of ethanolic extract of seeds of Artrocarpusaltilisagainst Alloxan induced Diabetes in experimental rats at dose dependent manner. Alloxan is selectively toxic to pancreatic β cells that produce insulin due to the accumulation of alloxan through the GLUT2 transporter. Though, Alloxan by itself is not toxic, but once it is infiltrated to the pancreatic b-cells through the GLUT2 transporter, alloxan is reduced to dialuric acid in the presence of different cellular

reducing agents. The presence of both Alloxan and its reduction product, leads to the establishment of redox cycle for generation of superoxide radicals ($O^{\cdot}$). (Jyoti M et al. 2002) Toxic action of alloxan on β cells is initiated by free radicals formed by redox reactions. The intolerable rise in oxidative agents provokes necrosis of pancreatic b-cells known to be vulnerable to redox imbalance. This suggests that Alloxandiabetogenicity is a free radical mediated process. (EvansJL et al. 2002, Karahan I 2005) Furthermore, Alloxan toxicity is related to animal death due to hypoglycemic fatal convulsions. This study shows that oral administration of ethanolic extract of seeds of Artrocarpusaltilisprevented the diabetogenic effect of Alloxan; this is possibly due to the presence of polar compounds, which act by inhibiting Alloxan-induced free radicals production.

The animals showing blood glucose range of greater than 200 mg dL^{-1} were used for the main study. The hyperglycemia was confirmed after 72 hours of Alloxan monohydrate administration (i.p.). It has been found that oral administration of test extracts at defined dose of 350 mg/kg b.w. caused a more significant potent reduction in blood glucose than other compounds in diabetic rats. This study reveals significant result of test groups, when compared with positive control (Alloxan 120 mg/kg) p.o. and standard Glibenclamide 10 mg/kg (p.o.). On day one to day three, the blood glucose measurements of diabetic rats not treated and those treated with the seed extract of A. altilisand glibenclami significantly ($p < 0.05$) elevated compared to the control (Table- 5 and Fig. 1). However, on the 7th day to the end of the study, the 15th day, there was no significance difference in the blood glucose measurements of diabetic rats treated with the seed extract of A. altiliscompared to the control (Table- 5).The rats treated with 250 and 350 mg/kg of the seed extract of A. altilison the 7th day showed a marked blood glucose reduction of 69.23% and 75.21%, respectively (Table- 5). Conversely, the reference drugs glibenclamide had a plasma glucose reduction of 58.18% (Fig. 1).

On the 15th day, the rats treated with 250 and 350 mg/kg of the seed extract of A. altilisshowed a significant glucose reduction of 73.00% and 77.36%, respectively, compared to the 68.54% glucose reduction of glibenclamide (Table 6).The effect of the seed extract of A. altilison the body weight of the rats is summarized in Table-7. The seed extract of A. altilisat doses of 250 and 350 mg/kg caused significant ($p < 0.05$) reduction in the weights of the rats from the 5^{th} to 15^{th} day of the study while 250 mg/kg of the extract caused no significant change in the weights of the rats except on the 11^{th} day when the reduction became significant ($p < 0.05$) compared to the control. The weights of the diabetic rats that were not treated reduced significantly ($p < 0.05$) from the 7^{th} to the 15^{th} day of the study (Fig.3).

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Author contribution

All author participated Equally**.**

Conflict of Interest

None

Fundig

None

Ethical Clearance

Taken from Institutional ethical committee.

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