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Research Articl

EVALUATION OF ANTIDIABETIC ACTIVITY OF *ANNONA SQUAMOSA LINN SEED* IN ALLOXAN – INDUCED DIABETIC RATS

Ravinder Sangala*, DevenderRao Kodati , Shashidher Burra, Jayaprakashreddy Gopu, Ajay Dubasi.

Department of Pharmaceutical Sciences, St.Peter's College of Pharmacy, Madikonda, Warangal-506 142.

ABSTRACT

Aim: A wide number of herbal products are employed in the treatment chronic disorder in human i.e. Diabetes mellitus, responsible for different complications, causes mortality, morbidity for their better efficacy and safety compare to synthetic medicine. In the present study indicate that the methanolic and ethanolic extracts 200 mg/kg b.wt. of seeds of Annona *squamosa* as significant hypoglycemic activity in both normal and Alloxan induced diabetic rats due to the presence of more than one anti hyperglycemic principles. At the 7th day of post administration of methanol and ethanol extracts, the percentage of blood glucose lowering potential was observed as 45.99% and 43.96% respectively; while standard Glibenclamide caused 59.21% reduction blood glucose in the untreated group appear to be higher than that in the treated group. Pharmacological and biochemical investigation will clearly elucidate the mechanism of action and will be help full in projecting this plant as a therapeutic target in diabetic research.

Keywords: Antidiabetic Activity, *Annona squamosa.Linn seed*, alloxan – induced diabetic rats.

INTRODUCTION

Medicinal plants have created the foundation of health care system throughout the world since the initial stage of humanity and still plant products are the major source drug/formulation in treatment of various diseases [1]. Herbal drug and phytoconstituents resembles safely and efficacy, they produces less/no side effect when compare to synthetic drugs. The use of plants in religious rituals as well as for magic and medicinal purposes is well known and widespread. In the modern era also most of the people believe the plants and phyto constituents are better choice to treat diseases than the allopathic drugs, even most of the drugs used in primitive medicine were instigated from plants [2,3].

Diabetes mellitus is one of the most common endocrine disorders, characterized by hyperglycemia with altered lipids, carbohydrates and protein metabolism. It occurs mainly due to absent or inadequate pancreatic insulin secretion, with or without concurrent impairment of insulin action. According to WHO, about 143 million people worldwide suffering from diabetes and the number may likely to double by the year 2030 [4,5]. Diabetes mellitus encountered as a major public health, produces different complications and also responsible for increased number of morbidity and mortality [6].

Two major types of diabetes mellitus are,

Type – 1: Insulin dependent diabetes mellitus (juvenile onset diabetes mellitus). About 5-10% of children and young adult with average age of 11 -12 years has no ability to produce insulin because of beta cells destruction in pancreatic islets.

Type – 2: Non insulin dependent diabetes mellitus (maturity onset diabetes mellitus)

About 80 – 90% people over 40 years of age are more prone to type – 2 diabetes. This occurs due to insulin insufficiency or impaired insulin secretion (females >males).

Corresponding Author: **Ravinder Sangala** Email: Davidraaz9773@gmail.com

MATERIALS AND METHODS

Materials: Alloxan monohydrates, Glibenclamide (MSN laboratories, ram nagar, hydrabad), Normal saline, 5% Tween 80 solution, 25% dextrose,5% dextrose, Distilled water.

Animals: Both sexes of wistar rats, weighing between 150-20 g, were used in the study [7]. They were maintained under standard laboratory conditions at ambient temperature. They were fed with standard pellet diet and water ad libitum. Animals were fasted for 18 hours before experiment and during the experiment they were withdrawn from food and water. The prior approval for conducting the experiments in rats was obtained from our Institutional Animal Ethical Committee.

METHODS

Soxhlet Extraction: Soxhalation is a process of continuous extraction in which the same solvent can be circulated through the extractor several times. The process involves extraction followed by evaporation of the solvent. The vapors of the solvent are taken to a condenser & the condensed liquid will be returned to the drug for continuous extraction. Soxhalet consists of a body of extractor attached with a side & siphon tube. The extractor from the lower side can be attached to distillation flask & the mouth of extractor is fixed to a condenser by standard joints. The crude drug power is packed as thimble is placed in the soxhlet apparatus. The diameter of thimble corresponds to the internal diameter of the soxhlet extractor. Few porcelain pieces are added into the flask to avoid bumping of the solvent. The vapours of solvent pass through the side tube and are condensed as liquid gradually increasing the levels of liquid in the extractor & siphon tube. A siphon is setup as the liquid reaches the point of return & the contents of the extractor chamber are transferred to the flask. The cycle of solvent evaporation & siphoning back can be continued many times without changing solvent to get efficient extraction.

In the present investigation, the extracts of powdered *Annona squamosa* seeds were prepared by sequential soxhalation extraction with different solvents in their order of polarity viz methanol ,ethanol finely powdered *Annona squamosa* seeds were packed in a thimble & inserted in the soxhlet apparatus, the extraction process with each solvent was continued for 24 hours, and the drug to solvent ratio of 1:3 was maintained after 24 hours the contents were filtered through whatmann No. 1

Anti-diabetic study

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted [11]. Alloxan is one of the usual substances used for the induction filter paper & sterilize muslin cloth which ever applicable. Filtrate concentrated under vacuum & dried in dessicator. Each time before extraction with next solvent, the powder material was air-dried. Finally, the extracts obtained by the two-extraction process were weighed [8-10].

Study in diabetic rats

Induction of Diabetes in rats: Rats were made diabetic by a single I.P. injection of 150mg/kg of Alloxan monohydrate dissolved in saline to overnight fasted animals. It is followed by 0.5 ml of 25% Dextrose after 2 hours of Alloxan and 5% Dextrose solution ad libitum for next 24hours.After 72 Hours of Alloxan, blood samples were withdrawn from rat tail vein and blood glucose levels were estimated in all animals. Animals with normal blood glucose level ≥ 200mg/dl (Diabetic) were selected for study. Antidiabetic activity study in diabetic rats using *Annona squamosa* seed extract:

Rats were divided into 5 groups. Each group contains 6 rats.

Group 1 - Normal control

Group 2 - Diabetic control (receive vehicle)

Group 3 - Diabetic+ Glibenclamide (10mg/kg b.wt)

Group 4 - Diabetic+ Methanol Extract of seed (200 mg/kg b.wt)

Group 5 - Diabetic+ Ethanol Extract of seed (200 mg/kg b.wt)

Blood samples were collected from rat tail vein and blood glucose levels are estimated using one touch glucometer on 3^{rd}, 5^{th}, and 7^{th} days.

RESULTS

The pharmacognostical studies were made on the seed of Annona squamosa, these observations will help in the botanical identification and standardization of the crude drug form and also to distinguish the drug from adultrants. Pharmacognostical evaluation designed to detect and check adultration and exhausted drug, absence of other parts of plant, presence of abnormal proportion of extrenious matter**.**

Preliminary tests were carried out for the presence or absence of phytoconstituents like Glycosides, Flavanoids, Saponins, acetagens, Alkaloids, Carbohydrates, Sterols, Proteins, Phenolic compounds and reducing compounds.

Results of Phytochemical tests of Methanol and Ethanol extract of *Annona squamosal seed:*

of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas [12]. Alloxan causes a massive reduction in insulin release by the destruction of b-cells of the islets of langerhans, thereby inducing hyperglycaemia Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose [13,14].

Effect of *Annona squamosa* seed extract on blood glucose level in diabetic rats: Ethanolic extract of *Annona squamosa* seeds was subjected to anti-diabetic activity in rats where alloxan monohydrate (150 mg/kg b.w., i.p.) used as the diabetogenic agent.A marked rise in fasting blood glucose level observed in diabetic control compare to normal control rats. Ethanolic extract (at 200 mg/kg) and methanolic extract (200mg/kg) of *Annona squamosa* exhibited a dose dependent significant anti-hyperglycemic activity on 3rd, 5th and 7th day post treatment. The extract dose of caused reduction in blood glucose level and the results were found statistically significant. The antihyperglycemic effect of ethanol extract at was found less effective than the reference standard, Glibenclamide. Glibenclamide produced a significant reduction in blood glucose compare to diabetic control.

Table 1. Phytochemical evaluation

Type of phytoconstituent	Ethanol Extract	Methanol extract
Alkaloids	++	++
Carbohydrates	++	++
Flavanoids	+	++
Glycosides	++	++
Saponins	+	++
Steroids & Terpinoids	+	+
Proteins	+	+
Fixed oils	++	++
Tannins	+	+

—: Absent; +: Slightly Present; ++: Moderately Present.

Table 2. Normal blood glucose levels of rats

Sl.No	Animal weight (in grams)	Normal blood glucose level(mg/dl)
1	165	88
2	170	100
3	180	100
4	160	110
5	168	109
6	172	99

Diabetic control blood glucose levels:

Table 3. Blood glucose levels(BGL) of Diabetic control rats

Sl. No.	Animal weight (in grams)	Normal blood glucose level(mg/dl)	After treatment with Alloxan mono hydrate(150mg/kg)	Blood glucose levels (BGL) (mg/dl)		
				3rd day	5th day	7th day
1	230	109	202	210	208	212
2	220	102	215	218	220	222
3	220	100	206	211	209	211
4	200	110	204	210	212	215
5	225	105	208	215	214	218
6	232	107	210	217	215	220

Table 4: Blood glucose levels (BGL) of Glibenclamide treated rats

Sl. No.	Animal weight (in grams)	Normal BGL (mg/dl)	BGL After treatment with Alloxan (150mg/kg)	BGL after the treatment with Std.		
				3rd day	5th day	7th day
1	225	105	315	202	158	130
2	240	110	310	201	156	127
3	230	112	312	207	153	125
4	245	102	310	205	155.2	126.5
5	233	109	313	209	152	127.8
6	242	106	309	212	157.1	126

Table 5: Blood glucose levels (BGL) of Methanolic seed extract treated rats

Sl. No.	Animal weight (in grams)	Normal BGL (mg/dl)	After treatment with Alloxan (150mg/kg)	BGL after the treatment with MSE(mg/dl)		
				3rd day	5th day	7th day
1	230	87	240	182	151.6	139
2	240	84	284	192	160	145
3	220	85	242	179	150.1	140
4	225	89	281	190	159.2	146
5	230	86	270	186	153	142
6	228	87	266	179	152	140

Table 6: Blood glucose levels (BGL) of Ethanolic seed extract (ESE) treated rats

S. No	Animal weight (in grams)	Normal BGL (mg/dl)	After treatment with Alloxan (150mg/kg)	BGL after the treatment with ESE (mg/dl)		
				3rd day	5th day	7th day
1	230	85	240	180	152	140
2	235	96	255	175	155.8	142
3	240	92	252	173	150	140
4	245	99	260	172	150.2	140.3
5	233	90	248	174	150	141.6
6	246	94	250	170.8	149.6	139.8

Figure 1. Treatment with standard drug (Glibenclamide)

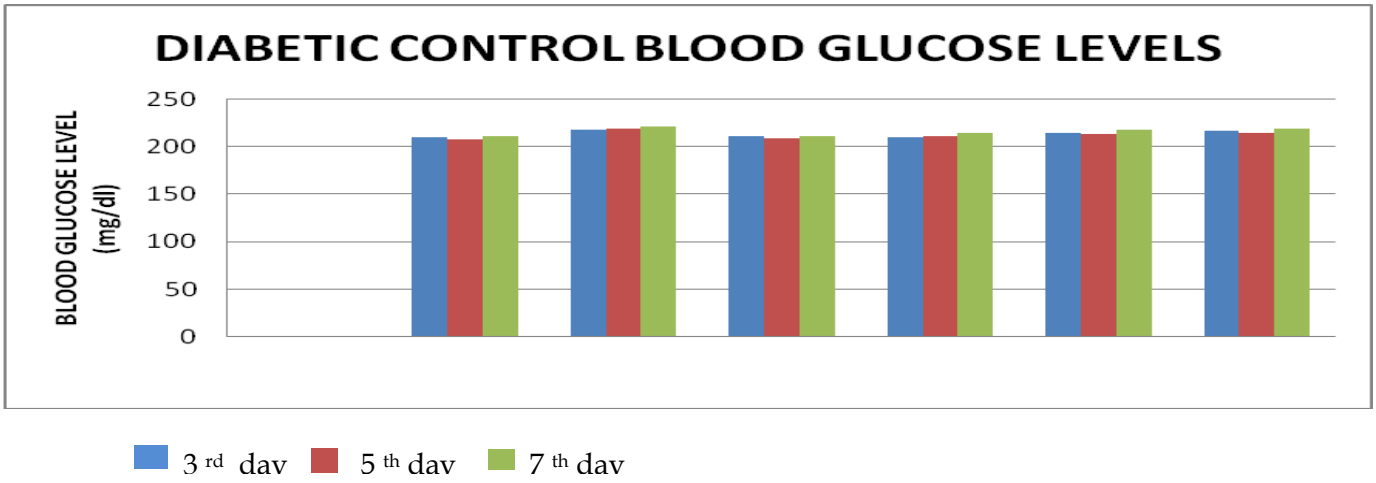
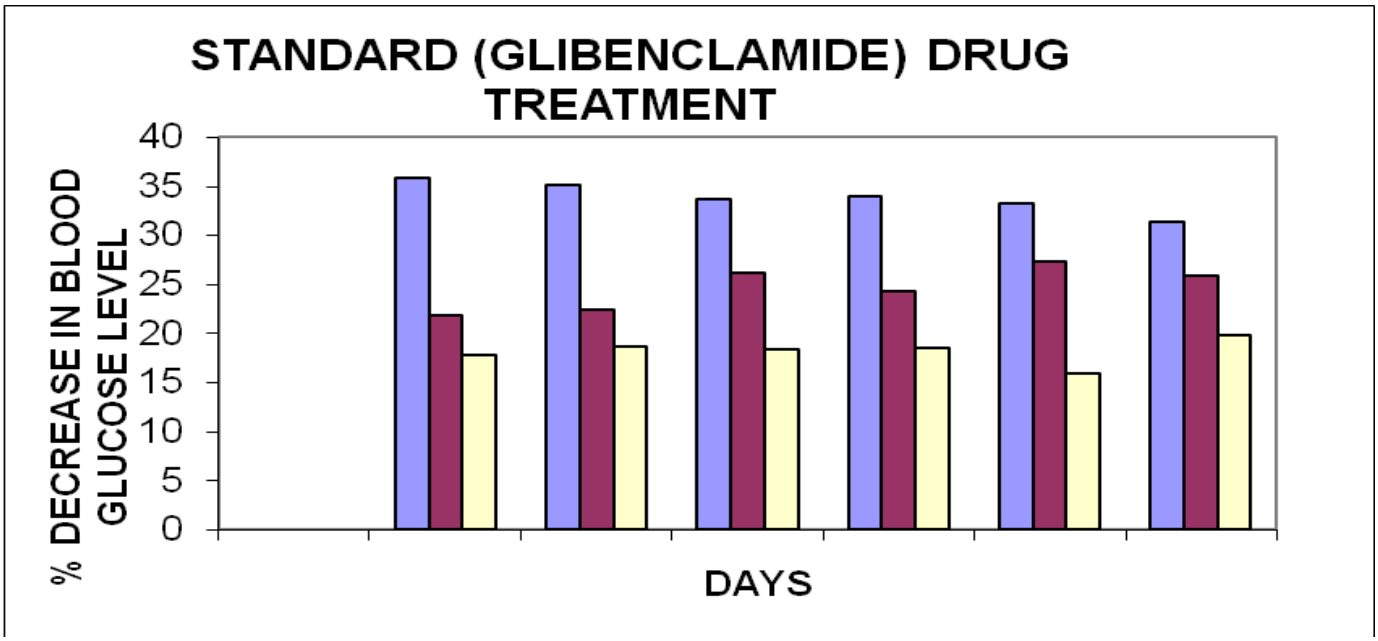
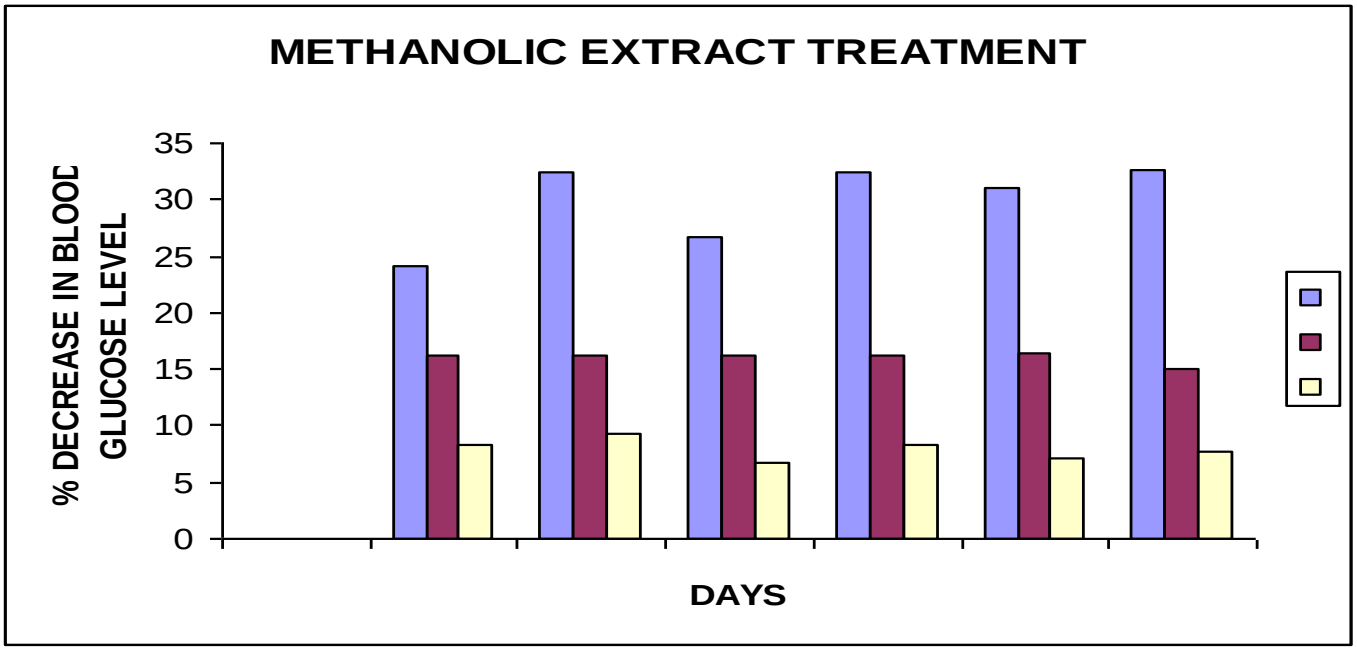


Figure 2.Treatment with Methanolic seed extract



: 3 rd day; 5 th day: 7 th day

Figure 3. Treatment with Methanolic seed extract



3 rdday: 5 th day; 7 th day

Figure 4. Treatment with Ethanolic seed extract

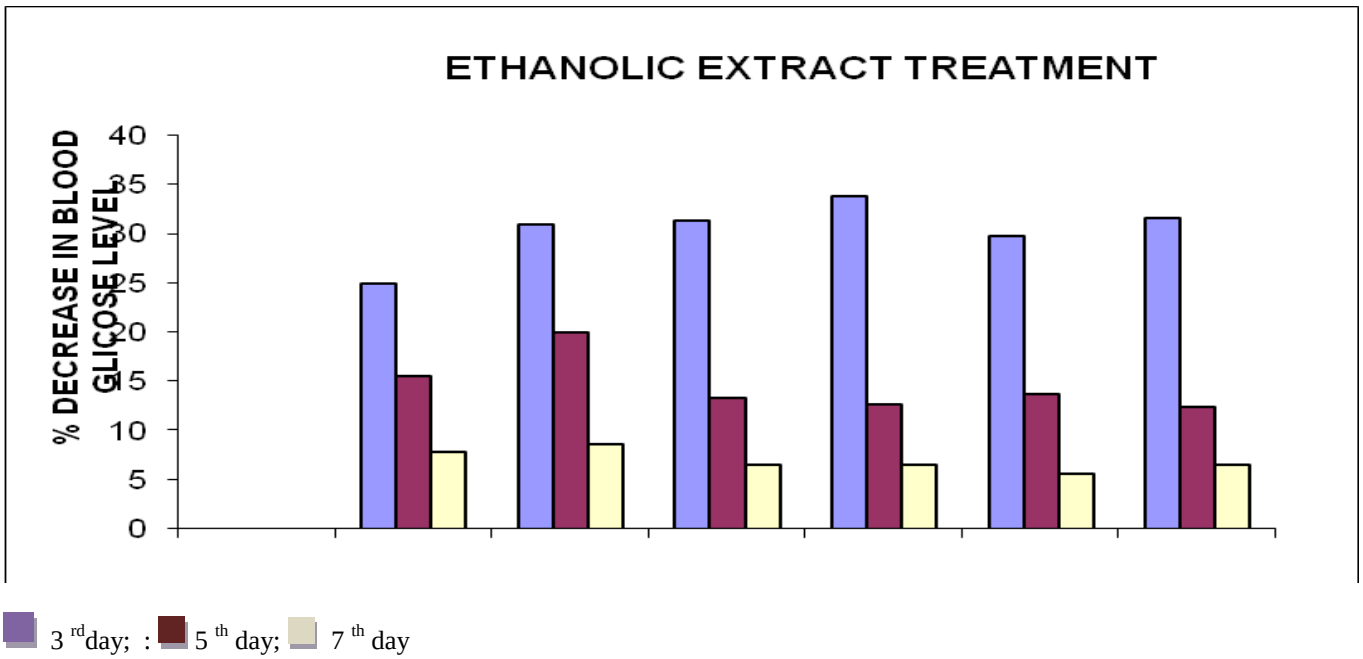
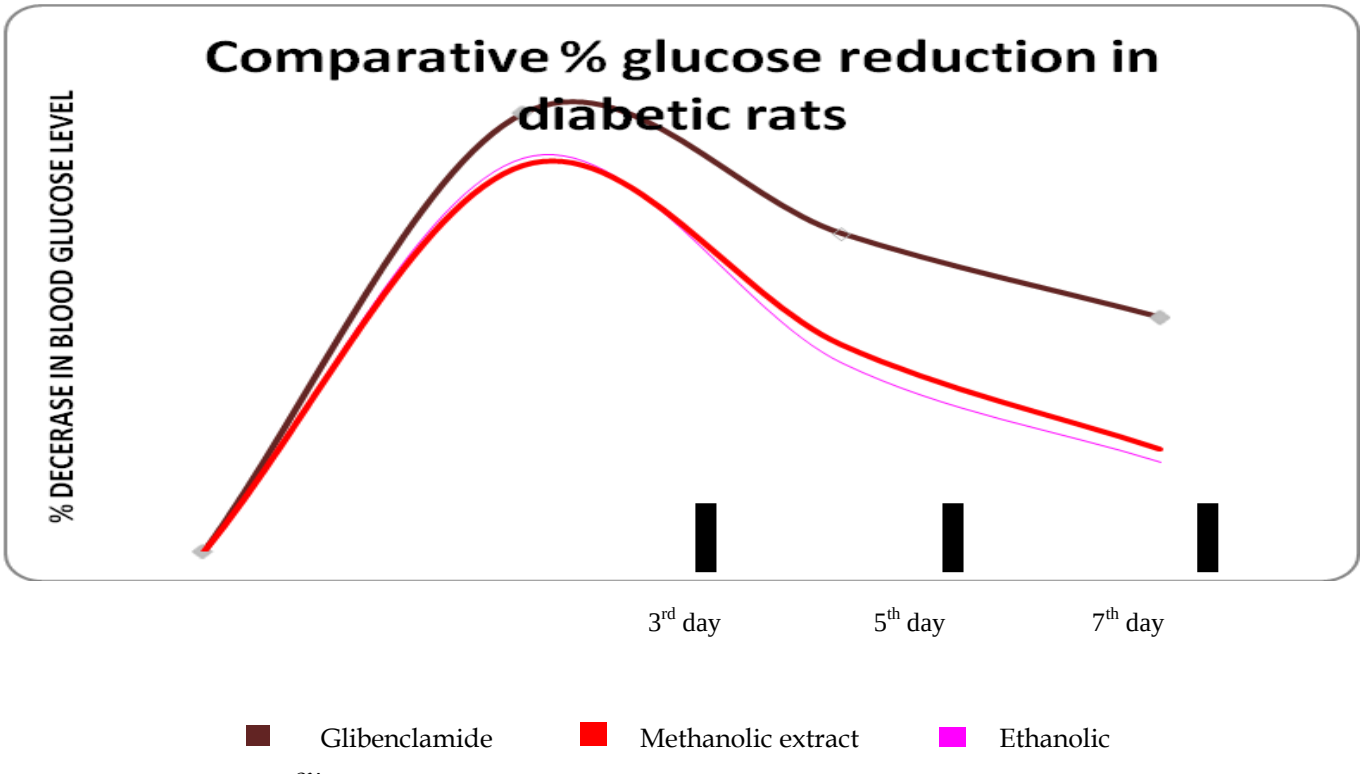


Figure 5. comparative %glucose reduction in diabatic rats



DISCUSSION

From the above results Crude methanol and ethanol extracts exhibited antidiabetic property in Alloxan – induced diabetic rats as evident from the findings. At the 7^{th} day of post administration of methanol and ethanol extracts, the percentage of blood glucose lowering potential was observed as 45.99% and 43.96% respectively; while standard Glibenclamide caused 59.21% reduction blood glucose in the untreated group appear to be higher than that in the treated group. Comparison of average values of BGL in treated and untreated (control group of alloxan induced diabetic rats suggest some favorable Antidiabetic effect of *Annona squamosa).* From the present experimental results it can be suggested that the methanol and ethanol extracts of *Annona squamosa* may exhibited dose dependent action in a similar mechanism as Glibenclamide i.e., by stimulation of surviving β – cells to release more insulin.

CONCLUSION

The results obtained in the present study clearly indicate that the Methanolic and Ethanolic extracts 200 mg/kg b.wt. of seeds of *Annona squamosa* as significant hypoglycemic activity in both normal and Alloxan induced diabetic rats. Flavonoids, saponins, acetagenins, alkaloids and phenolics are known to be bio active antidiabetic principles. The antidiabetic effect of Methanolic and Ethanolic extracts of seeds of *Annona squamosa. Linn* may be due to the presence of more than one anti hyperglycemic principles mentioned above. Further pharmacological and biochemical investigation will clearly elucidate the mechanism of action and will be help full in projecting this plant as a therapeutic target in diabetic research.

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