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Evaluation Of Antihyperglycemic Effect Of Aqueous Extract Of Leaves Of *Annona squamosa* In Streptozotocin Induced Diabetic Rats.

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ABSTRACT

The aim is to evaluate the antihyperglycemic effect of *Annona squamosa* leaf extract in streptozotocin induced diabetic rats by tail venipuncture method. 24 adult male albino rats weighing 150-200g were selected from central animal house, Madurai Medical College, Madurai. Initially, 24 albino rats were divided into 4 groups of 6 animals each. Group I received normal feed. Group II received Tab. Glibenclamide1mg/kg orally. Group III and Group IV received *Annona squamosa* leaf extract 300mg/kg and 600mg/kg orally for 14days.The blood glucose level was monitored on day 1, 7 and 14 by tail vene puncture method. On day 7 and day 14, there was a significant fall in blood glucose level in the *Annona* treated groups, when compared with the control ($p < 0.001$). The values of Test 2, were comparable with that of standard group. A post hoc analysis was conducted, which showed the statistical difference was more with standard group > Test group 2 > Test group 1, when compared with the control group. It was observed that *Annona* leaf extract at 300 mg/kg and 600 mg/kg produce statistically significant reduction in blood glucose level in streptozotocin induced diabetic rats when compared with control group. Increased mRNA expression of GLUT4 in peripheral tissues, the insulin releasing property, free radical scavenging property, inhibition of intestinal absorption of glucose and inhibition of PTP1B, might be possible mechanisms for the antihyperglycemic activity of *Annona squamosa*.

Keywords: *Annona squamosa*, Glibenclamide, antihyperglycemic, tail vene puncture.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder with features of chronic hyperglycemia, that occurs due to defective insulin secretion or resistance to insulin action or both. The long-term effects include end organ damage especially to the eyes, kidney, heart, blood vessels and nerves [1]. The global prevalence of diabetes among adults is estimated to be 6.4% in 2010 and is extrapolated to increase to 7.7% by 2030. As an emerging epidemic in India, it was estimated that 62.4 million are diabetic in 2011 and it is proposed to increase to 101.2 million by 2030 [2].

Several theories have been postulated for pathogenesis of diabetes. The glycoxidation hypothesis proposed that pentosidine and carboxymethyl lysine, which are a subclass of advanced glycosylation end products that cause oxidative damage to long lived proteins in diabetes [3].

The free radical theory connotes that the reactive oxygen species leads to cellular damage through stress sensitive pathways such as nuclear factor kappa (NF-KB) , mitogen activated protein kinase (p38 MAPK) and hexosamine pathway which is responsible for onset of late complications of diabetes [4].

Of all the treatment modalities for diabetes, life style intervention is promoted as an effective means to prevent or delay the incidence of diabetes and its complications, thereby reducing the public health burden [5]. Pharmacotherapy of diabetes commonly includes usage of sulfonyl urea's, biguanides and thiazolidinediones. But all these synthetic oral drugs are laden with adverse events on prolonged use [6].

This has led to an exponential growth in the field for research in herbal remedies, that have antihyperglycemic, and anti-oxidant potential, with a minimum of adverse effects.

Annona squamosalinn [7], one such plant with antidiabetic potential of the family Annonaceae, is commonly cultured in Thailand and its origin is from South America. However, it is commonly found in India and is grown in gardens for its ornamental values and fruits. It is a small ever green tree (or) a shrub of 7 meters in height, that bears edible fruits called " sugar apple". It is also known as custard apple, sharifa or sitaphalam [8].

The plant is reported to contain glycosides, alkaloids, flavanoids, tannins, phytosterols, anonaine, aporphine, glaucine, isocorydine, norcorydine, coryeline. The leaves are rich in anonine, borneol, camphene, β-caryphyllene, eugenol, farnesol, geraniol, hexacontanol, isocorydine, higemamine, limonine, linalool acetate, menthone, methyl salicylate, β-sitosterol, thymol, rutin, n-triacontanol [9]. The presence of acetogennins were considered responsible for antimalarial, Immunomodulatory, Cytotoxic activities.

The Presence of diterpenes was found to possess, Anti-HIV activity, Anti platelet aggregatory activity. Flavanoids were reported to have antimicrobial, pesticidal activities.

The aqueous extract of leaves was reported to attenuate hyperthyroidism. It was used by tribes to manage diabetes. They have astringent properties. Various parts of the plant were proposed to have mollusicidal activity against Schistosoma species. The flavanoids in the leaf extract have antioxidant activity which at times, confers a hepatoprotective effect. They possess larvicidal activity against Anopheles stephensi and they are also active against helminthes. The acetogenins were considered responsible for the above mentioned effects. As a novel attempt to cut down the complications and cost of oral hypoglycemic agents and provision of better glycemic control in patients with diabetes, this study has been performed to evaluate the antihyperglycemic effect of the aqueous extract of the leaves of *Annona squamosa (linn)* in diabetic rat models. Of all the theoretical models, the most feasible, reliable method is Streptozotocin [10] is an antimicrobial agent and has historically been used as an alkylating agent. Rakieten first described the diabetogenic potential of STZ. It is a 2 deoxy 2 (methyl nitrosoamino) carbonyl – amino) D- glucopyranose. It acts as a nitric oxide donor and causes DNA alkylation. Its β cell specificity is due to its selective cellular uptake and accumulation. Streptozotocin is a less lipophilic nitrosourea analogue, getting inside the β cells via GLUT 2 glucose transporter. The methyl nitrosourea moiety, causes transfer of the methyl group of streptozotocin to the DNA of host cell, resulting in fragmentation of DNA. Additional protein glycosylation and overstimulation of poly ADP – ribose polymerase causes cellular depletion of ATP and NAD, ultimately resulting in necrosis of the host cells. A minor mechanism of action may be due to generation of superoxide and hydroxyl radicals during the hypozanthine metabolism

thereby accelerating the process of cellular necrosis. A single intraperitoneal dose of 40-70 mg /kg is commonly used to induce diabetes in Wistar rats. The stability is best maintained at a pH of 4-4.5. Hence the STZ is better reconstituted in a citrate buffer and ice-cold saline preparation, freshly prepared and used within 30 minutes.

Aim

To study the antihyperglycemic activity of aqueous extract of leaves of Annona squamosa in Streptozotocin induced diabetic rats.

Methodology

The aqueous extract of leaves of Annona squamosa were evaluated for their antihyperglycemic effect, in adult male albino rats. The study was done in the Central animal house, Institute of Pharmacology, Madurai Medical College, Madurai, after obtaining approval from The Institutional Animal Ethical Committee of Madurai Medical College.

Animals Used

Twenty four, inbred adult male albino rats of 150 – 200 g, from Central animal house, Madurai Medical College were utilized in this study. The rats were equally split into four groups. Each group had six animals. One group was kept as control, one as standard. The remaining two groups were treated with the plant extract.The animals were given pellet feed and water according to their needs. Each group of animals were housed separately and distinctly marked with picric acid.

Streptozotocin

Streptozotocin was procured by HI Media Research Laboratories Pvt. Ltd.

All the groups of rats received injection streptozotocin intraperitoneally as a single dose of 50mg/kg.

Glibenclamide

Glibenclamide, a second generation sulphonyl urea was given to the standard group at an oral dose of 1 mg/kg/day.

Collection Of Blood Samples

The rats were kept in the restrainer. Lateral veins were located and xylol was applied to make the vein prominent. After disinfecting with spirit, 0.2 ml of blood was collected using a 22-gauge needle.

Method Of Glucose Estimation

Glucometer was used to detect the blood sugar levels. A drop of blood, withdrawn by tail venipuncture method, was directly placed on the strip.The results were displayed on screen within 15 seconds.

Extraction Procedure

Annona squamosa Leaf Aqueous Extract

The crude extract was prepared in the Pharmacognosy department of Madurai Medical College. Leaves were washed well with water.The fresh /air-dried leaves (25°C for 5 days in the absence of sunlight)were extracted in 1 litre of boiling water for 2 hours. The concentrate obtained was dark brown. It was cooled and filtered using Whatman No.1 filter paper. The filtrate was centrifuged and the sediment was discarded .The supernatant extract was concentrated and used for the study. Each day, the necessary amount of extract was dissolved in distilled water and administered orally.

Glibenclamide (1mg/kg) was similarly dissolved in distilled water and given daily. The standard drug as well as the test extracts of Annona squamosa were given orally using oral feeding tube and the treatment duration was for 14 days.

Oral Feeding Technique

A 16 Gauge feeding tube with a blunted tip was used. The tube was attached to 1 ml syringe which contained the drug to be given. The animals were handled with utmost care and held by the nape of their neck. The oral feeding tube was inserted laterally through the interdental space, and by gentle rotations it was placed in the oesophagus. After ascertaining the desired level, the drug was gently pushed inside.

METHODOLOGY

The study followed the guidelines of CPCSEA. After overnight fasting , the blood glucose level was observed for all the rats by tail venipuncture method. The baseline values were normal. All the rats were injected intraperitoneally with 50 mg/kg of Streptozotocin. Diabetic status of the rats were estimated after 3 days of streptozotocin injection, by repeat measurement of blood glucose levels. The animal was proclaimed diabetic if the blood sugar was > 250 mg/dl. The rats were split into groups of six and housed as control, standard, test 1 and test 2 groups. The control group received pellet diet and water. The standard group received the drug Glibenclamide at the dose of 1 mg/kg/day orally. Test 1 and test 2 groups received Annona squamosa leaf extract in the dose of 300 mg/kg and 600 mg/kg respectively.

GROUP STUDY TREATMENT

GROUP	STUDY	TREATMENT
I	Control	Normal feed and water
II	Standard	Normal feed and water +Tab Glibenclamide (1 mg/kg) oral
III	Test -1	Normal feed and water + aqueous extract of leaves of Annona squamosa (300 mg/kg) oral
IV	Test -2	Normal feed and water + aqueous extract of leaves of Annona squamosa (600 mg/kg) oral

The blood glucose level were estimated at baseline and on Day 1, Day 7 and Day 14 and the results are tabulated. ANOVA was used as the statistical test to detect any significant difference between the groups.

RESULTS

After baseline measurement of blood glucose levels in non diabetic rats, Streptozotocin intraperitoneally in a dose of 50mg/kg was given to all rats. After 72 hours, all the animals had blood glucose values >250 mg/dl. The first group received pellet diet and served as control. The standard group received Tab Glibenclamide 1mg/kg orally. The third and fourth groups received aqueous extract of the leaves of *Annona squamosa* in graded doses of 300 and 600 mg/kg respectively. The blood sugar levels of the rats on Day 1,7 and 14 are tabulatedin Tables 1,2,3 respectively and the graphical representation for the same is depicted in Figures 1,2,3.

Control Group

The mean blood glucose values of diabetic rats of the control group were 454.55±25 on day 1 ,484.1±20 on day 7 and 453.1± 12.57 on day 14 , as shown in Table No.4

Standard group

The mean blood glucose values of diabetic rats in the standard group after the administration of Glibenclamide were 557.6±22 on day 1 ,394±25 on day 7 and 94.6±15.4 on day 14 as shown in Table No.4.

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Test Group 1 (Annona squamosa extract 300mg/kg)

The mean blood glucose values of rats that received Annona squamosa extract of 300mg/kg were 582.1±11 on day 1 , 409±21 on day 7 and99.6±18 on day 14 as shown in Table No.4.

Test Group 2 (Annona squamosa extract 600mg/kg)

The mean blood glucose values of rats after administering Annona squamosa extract of 600mg/kg were 577 .1± 17 on day 1 , 398.3±35 on day 7 and 96.5±15 on day 14 as shown in Table No.4.

ANOVA was calculated between the groups and it showed no significant difference at their baseline values .The analysis of variance conducted after 14 days of treatment showed a significant difference ($p < 0.001$) between the groups. Figure 4 shows the blood glucose levels of the control group of diabetic rats during the study duration. The figure 5 and 6 compares the blood glucose levels of the test 1 group and test 2 group with the control, correspondingly and figure 7 compares the blood glucose levels of the treatment groups with the control group of diabetic rats.

A post hoc test conducted on day 14 showed the difference was significant in the standard group >annona 600mg/kg group >annona 300 mg/kg group, when compared with the control.

Table 1: Day one blood glucose levels in diabetic rats (mg/dl)

S. No.	Control	Standard	T1-300mg/kg	T2-600mg/kg
1	489	523	589	587
2	420	554	574	593
3	436	545	592	589
4	465	560	565	547
5	445	586	594	564
6	472	578	579	583

Figure 1

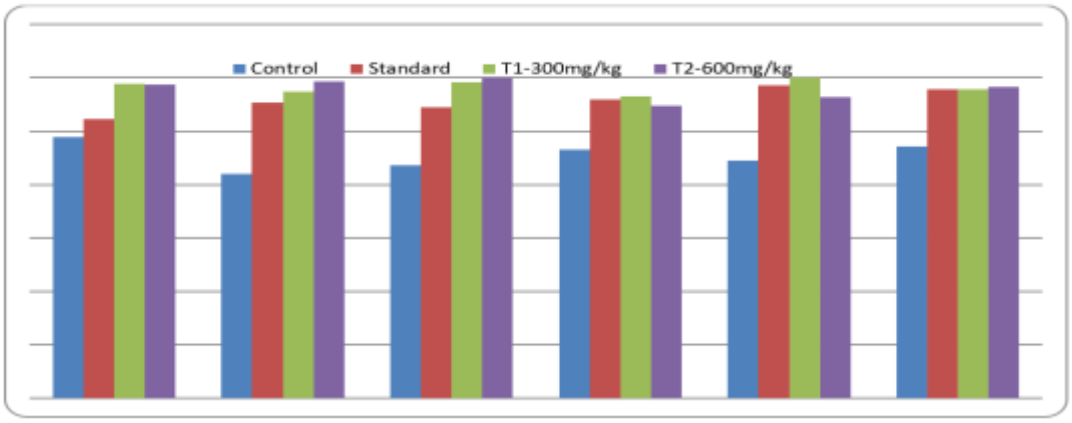

Table 2

S. No.	Control	Standard	T1-300mg/kg	T2- 600mg/kg
1	453	354	424	385
2	476	410	443	364
3	488	425	393	370
4	517	378	398	451
5	481	395	410	435
6	490	402	387	385

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Figure 2: The following graph was formulated from day 7 blood glucose values

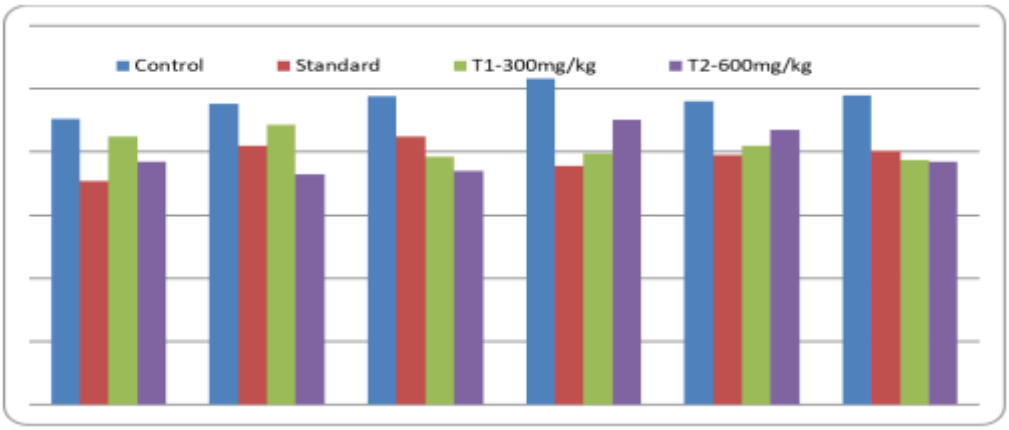

Table 3: **Day 14 blood glucose levels in diabetic rats (mg/dl)**

S. No.	Control	Standard	T1-300mg/kg	T2- 600mg/kg
1	435	98	90	114
2	446	102	77	103
3	459	86	131	85
4	447	117	105	74
5	464	71	97	93
6	468	94	98	110

Figure 3

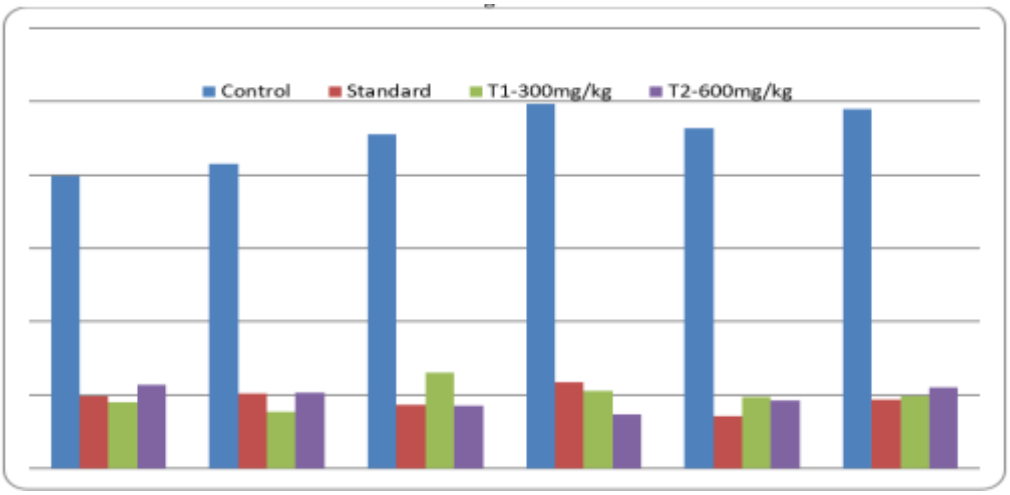

Table 4 -Blood Glucose Levels in Diabetic Rats in mg/dl

Group	Day 1	Day 7	Day 14
Control Normal pellet diet	454.55±25	484.1±20	453.1±39.7
Standard -Glibenclamide	557.6±22	394±25	94.6±15.4
Test 1-Annona squamosa extract 300 mg/kg	583.1±12	409±21	99.6±18
Test 2-Annona squamosa extract 600 mg/kg	579±19	398.3±35	96.5±15

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Figure 4

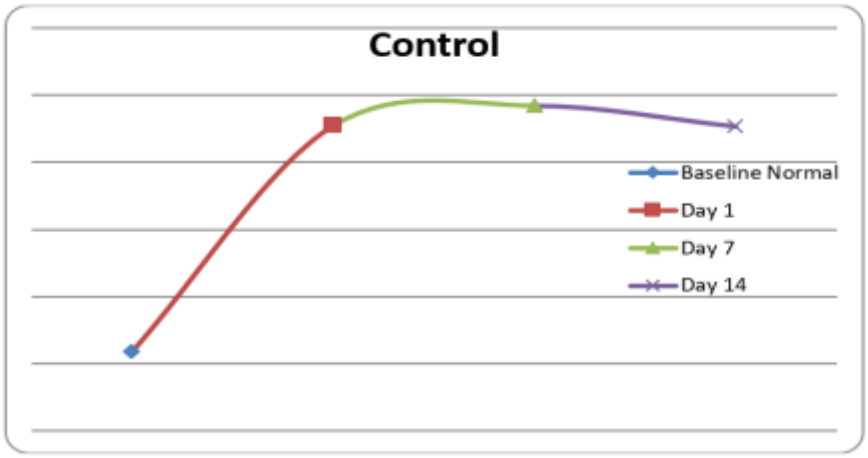

Figure 5

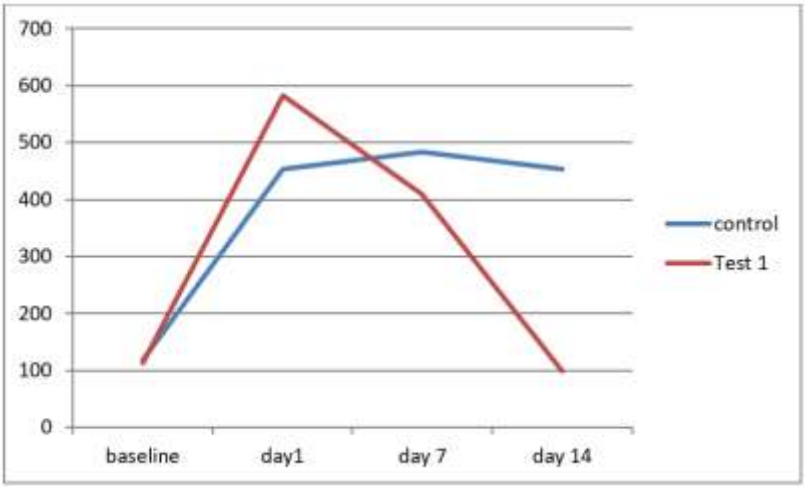

Figure 6

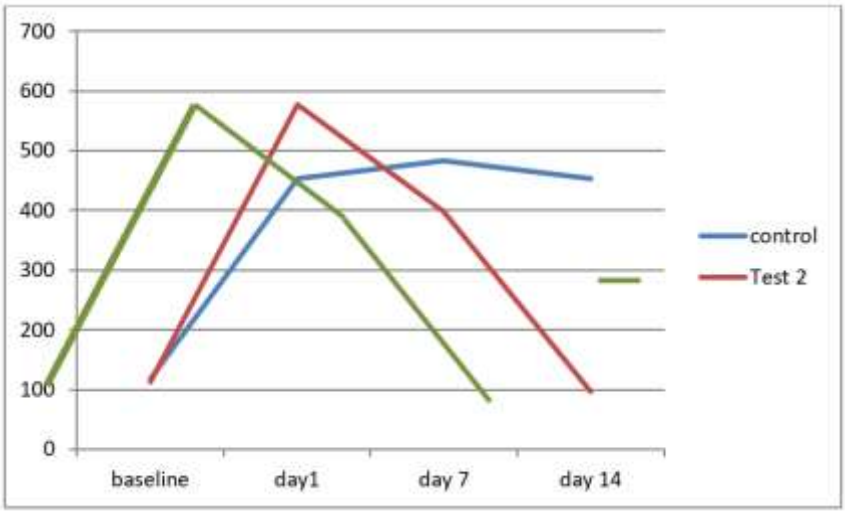

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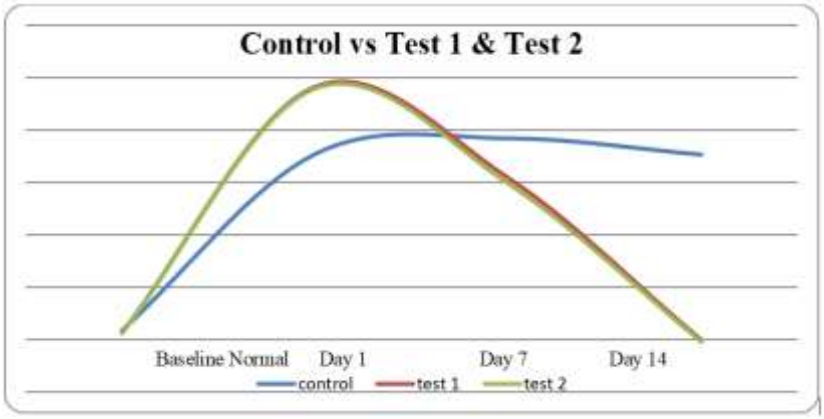
Figure 7



DISCUSSION

Diabetes is a global endocrine disorder, which is rapidly emerging as an "epidemic" in developing countries like India, which is being converted into a world capital for this disease. In the long run, this disease affects the quality of life and curtails life span. The comprehensive management of diabetes includes diet, life style intervention, along with pharmacotherapy, like parenteral insulin (or) usage of oral anti diabetic agents.Kaleem et al (2008), reported that the activity of the leaves of *Annona squamosa* might be through an enhancement of insulin release from beta cells of pancreas or it may be due to increased GLUT4 uptake of glucose from plasma to peripheral tissue. An increase in the levels of insulin and C-peptide were also noticed in the groups treated with plant extract, when compared with the sham group. It further showed an increase in the serum total protein and albumin levels, because of incorporation of the amino acids into proteins, by insulin [11]. Gupta et al, further suggested that the aqueous extract of leaves of *Annona squamosa*, increases insulin release even from partially destroyed pancreatic cells, by probably regenerating the beta cells (or) release of stored insulin from granules [12]. It was suggested to improve glucose tolerance and the extract reduced the levels of total cholesterol, LDL, VLDL in diabetic animals and also increased the levels HDL cholesterol.

In vitro studies from aqueous extract was shown to inhibit the glucose 6 phosphatase enzyme activity, thereby preventing glycogenolysis. The aqueous extract also slightly inhibited (18.1%) the intestinal absorption of glucose in rats.

Chronic hyperglycemia stimulates ROS formation through oxidative phosphorylation, NAD(P) H oxidase, lipooxygenase, cytochrome P450 monooxygenase, glucose auto oxidation. In vitro studies, proved that the leaves of *Annona squamosa*, have a free radical scavenging activity of 1,1, - diphenyl -2-picrylhydrazyl (DPPH) radical. The oxygen radical absorbance capacity (ORAC) assay, also quantitated the inhibition percentage of free radicals and the length of inhibition time in *Annona* extracts, which were proven significant [13]. In vitro studies on hexane extract of *Annona* leaves, showed inhibition of PTP1B (Protein tyrosine Phosphatase 1B) in a dose dependant manner. The normal function of PTP1B is negative regulation of signaling of insulin. This is achieved through dephosphorylation of IR-beta (Insulin receptor beta) and IRS-1 (insulin receptor substrate 1). The inhibition of PTP1B causes disinhibition of insulin secretion, thereby activating downstream cellular events and increasing P13 kinase dependant increase in glucose uptake into the cells. The in vitro study was followed by treatment of ob/ob mice with the herbal extract, which improved glucose tolerance and lowered the triglyceride levels [14]. A further fractionation of potential bio active molecules might be needed to further elucidate the possible links in mechanism of action of *Annona*.

In summary, increased mRNA expression of GLUT4 in peripheral tissues, the insulin releasing property, free radical scavenging property, inhibition of intestinal absorption of glucose and inhibition of PTP1B, might be responsible for the antihyperglycemic activity of Annona, leading to a statistically significant fall in the fasting blood glucose levels.

CONCLUSION

In this study, annona squamosa leaf extract which was widely used in folklore medicine for several ailments was evaluated for its antihyperglycemic effect in albino rats. It was observed that annona leaf extract at 300 mg/kg and 600 mg/kg led to a statistically significant fall in blood glucose level in diabetic rats when compared with the control group. Fractionation of the active principle and further studies on animals of higher phylogenetic scale is needed in the near future, to elucidate the mechanism of action and confirm the antihyperglycemic activity of Annona squamosa.

REFERENCES

[1] American Diabetes Association .Diagnosis and classification of diabetes mellitus. Diabetes Care 2010; 33(Supplement 1): S 62 -69.

[2] Ambrish Mithal, et al. Prevalence of dyslipidemia in adult Indian diabetic patients: A cross sectional study (SOLID).Indian J Endocrinol Metab 2014; 18(5): 642–647.

[3] John W Baynes, Suzanne R Thorpe. Role of Oxidative Stress on Diabetic Complications A New Perspective on an Old Paradigm. Diabetes 1999; 48 :1-9.

[4] Joseph L. Evans , Ira D Goldfine , Betty A.Maddux , and Gerold M.Grodsky.Are Oxidative Stress – Activator Signaling Pathways Mediators of Insulin Resistance and β- Cell Dysfunction ? Diabetes 2003; 52 :1 -8.

[5] Diabetes Prevention Program Research Group. Reduction In The Incidence Of Type 2 Diabetes With Lifestyle Intervention Or Metformin. N Engl J Med 2002 ;346(6) :393 -403 .

[6] Ahmed SK, Chakrapani Cheekavolu, Naveen Alasyam, Sunil M. Evaluation of Hypoglycemic property of Gurmar (Gymnema Sylvestre) Leaves Methanolic Extract (GLSME) in Streptozotocin induced Diabetic Albino Rats . Journal Of Medical Science And Clinical Research 2017 5(12): 31753 – 31760.

[7] Neha Pandey, Dushyant Barve. Phytochemical and Pharmacological Review on *Annona squamosa* Linn.International Journal of Research in Pharmaceutical and Biomedical Sciences 2011; 2(4): 1404 -1412 .

[8] Mohammad Zahid, Mohd Mujahid, Prashant Kumar Singh, Sana Farooqui, Kuldeep Singh, Shahla Parveen and Muhammad Arif. *Annona Squamosa Linn*.(Custard Apple): An Aromatic Medicinal Plant Fruit With Immense Nutraceutical And Therapeutic Potentials. International Journal of Pharmaceutical Sciences and Research 2018; 9(5) :1745 – 1759.

[9] Binit Kumar, Shirish Dongare, E. Anand. Antidiabetic agents. Drugscreening methods. In: S K Gupta. 3rd edition. New Delhi: Jaypee The health sciences publishers; 2016. p. 613-644.

[10] S Lenzen. The mechanisms of alloxan- and streptozotocin-induced diabetes . Diabetologia 2008; 51: 216–226.

[11] Rajesh Kumar Gupta, Achyut Narayan Kesari, Geeta Watal, P. S. Murthy, Ramesh Chandra, Kapil Maithal and Vibha Tandon. Hypoglycaemic and antidiabetic effect of aqueous extract of leaves of *Annona squamosa* (L.) in experimental animal . Current Science 2005; 88(8): 1244 -1254.

[12] Mishra Shanti Bhushan, Rao Ch V, Ojha SK, Vijayakumar M, Verma A. An analytical review of plants for anti diabetic activity with their phytoconstituent & mechanism of action .International Journal Of Pharmaceutical Research And Sciences 2010; 1(1): 29 -46.

[13] Joseph Alex Davis, Suchitra Sharma, Shivani Mittra1, S. Sujatha, Anil Kanaujia, Gyanesh Shukla, Chandrakant Katiyar, B.S. Lakshmi, Vinay Sheel Bansal, Pradip Kumar Bhatnagar.Antihyperglycemic effect of *Annona squamosa* hexane extract in type 2 diabetes animal model: PTP1B inhibition, a possible mechanism of action?. Indian Journal of Pharmacology 2012; 44 (3): 326 -332 .

[14] Aaishwarya B Deshmukh, Jayvadan K. Patel. Aqueous extract of *Annona squamosa* (L.) ameliorates renal failure induced by 5/6 nephrectomy in rat. Indian Journal of Pharmacology 2011; 43(6): 718 – 721.