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Research Article

Antidiabetic and Antihyperlipidemic Activity of *Annona Squamosa* Fruit Peel in Streptozotocin Induced Diabetic Rats

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ABSTRACT
The present study was undertaken to investigate the effect of various extracts of fruit peel of *Annona squamosa* on blood glucose and lipid profile in streptozotocin induced diabetic rats. Different extracts (Petroleum ether, Ethyl acetate and Alcoholic) of *Annona squamosa* fruit peel was administered orally (250mg/kg body weight) for 21 days. The effects of different extracts of *Annona squamosa* on blood glucose and lipids profile were estimated in streptozotocin induced diabetic rats. The effects were compared with glibenclamide. The treatment with alcoholic extracts of *Annona squamosa* fruit peel and Glibenclamide resulted in a significant reduction of blood glucose. The alcoholic extract of *Annona squamosa* also resulted in a significant decrease in lipid profile. The decreased blood glucose and lipid profile clearly showed the antidiabetic and antihyperlipidemic effect of *Annona squamosa* fruit peel extract.

Key words: *Annona squamosa*, Glibenclamide, Streptozotocin, Antidiabetic activity, Antihyperlipidemic activity.

INTRODUCTION

Diabetes mellitus is a syndrome, initially characterized by a loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting impaired metabolism of glucose and other energy yielding fuels such as lipid and proteins[1]. Experimental diabetes in animals has provided considerable insight into the physiologic and biochemical derangement of the diabetic state. Many of the derangement have been characterized in hyperglycemic animals. Significant changes in lipid metabolism and structure also occur in diabetes[2]. In these cases the structural changes are clearly oxidative in nature and are associated with development of vascular disease in diabetes[3]. In diabetic rats, increased lipid peroxidation was also associated with hyperlipidemia[4]. In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease[5]. Many traditional plant treatments for diabetes are used throughout the world. Plant drugs[6] and herbal formulation[7,8,9] are frequently considered to be less toxic and free from side effects than synthetic one. In general, there is very little biological knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc., that are frequently implicated as having antidiabetic effects[10].

Annona squamosa Linn. (Annonaceae) is used in traditional medicine and is pharmacologically active. The roots, leaves, fruits, seeds & bark of plant has multiple uses. The fruits are very high in calorific value & are rich source of minerals & vitamins. The seeds are powerful insecticides & powdered seeds are used for removing head lice. Seeds also used as abortifacient & insecticidal. The leaves are shown to have anti-daibetic properties. It is also known for its hepato-protective powers. Fruits are sweet, haematinic, cooling, act as sedative, stimulant & function as expectorant & tonic[11]. *Annona squamosa* are traditionally used for antidiabetic and antihyperlipidemic activity[12].

The present investigation was undertaken to study the effects of fruit peel extracts of *Annona squamosa* on blood glucose and lipid profile in streptozotocin induced diabetic rats. The effect produced by different extracts of fruit peel of *Annona squamosa* on different parameters were compared with glibenclamide, a reference drug.

MATERIAL AND METHODS

Animals: Healthy wistar albino rats of either sex weighing 150-170g were used for this study. Before starting the experiment, the animals were acclimatized to the laboratory conditions for a period of 2 weeks at an ambient temperature (24±2 ºC) and relative humidity (40-60%). The light - dark cycle was followed. The animals were fed with standard laboratory diet and water *ad libitum*. The animals were fasted for overnight before the study but had free approach to water. All the experimental procedure and protocols were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) and all the experiments were carried out by following the guidelines of CPCSEA.

Plant Material & Extract Preparation: The fruits of *Annona 1lavonoid* were collected from local market of Jaipur and were authentificated from Department of Botany,

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Table I: Grouping of animals [15,16,17]

Group No.	Description
1	Served as normal control (Received normal saline 0.5 ml/kg body weight).
2	Served as diabetic control (treated with STZ dissolved in 0.1M sodium citrate buffer pH 4.5 at a dose of 50 mg/kg body weight).
3	Served as reference standards (glibenclamide, 500 µg/kg body weight orally).
4	Diabetic rats given pet ether extract of *Annona squamosa*, 250 mg/kg body weight which is prepared in 1 % CMC and was given orally.
5	Diabetic rats given ethyl acetate extract of *Annona squamosa*, 250 mg/kg body weight which is prepared in 1 % CMC and was given orally.
6	Diabetic rats given alcoholic extract of *Annona squamosa* , 250 mg/kg body weight which is prepared in 1 % CMC was given orally.

Table II: Cholesterol, triglycerides & HDL reagents composition

Reagent No.	Name of Reagent	Composition
1	Cholesterol reagent	Good buffer (pH 6.7) – 50 mM/L Cholesterol esterase - ≥100U/L Cholesterol oxidase - ≥ 50 U/L Peroxidase > 3KU/L 4-Aminopterine 0.4 mM/L Stabilizers q.s.
2	Cholesterol standard	Cholesterol – 200 mg/dl Preservative - q.s. Stabilizer- q.s.
3	Precipitating reagent	PEG 6000 – 200mM/L Preservative - q.s. Stabilizer- q.s.
4	HDL Cholesterol standard	Cholesterol – 50 mg/dl Preservative - q.s. Stabilizer- q.s.

Table III Procedure of cholesterol estimation

Sample	Serum	Reagent 2	Reagent 1
Blank	-	-	1000 µl
Standard	-	10 µl	1000 µl
Test	10 µl	-	1000 µl

Table IV Procedure of triglycerides estimation

Sample	Serum	Reagent 2	Reagent 1
Blank	-	-	1000 µl
Standard	-	10 µl	1000 µl
Test	10 µl	-	1000 µl

Table V Procedure of HDL estimation

Sample	Serum	Reagent 2	Reagent 1
Blank	-	-	1000 µl
Standard	-	100 µl	1000 µl
Test	100 µl	-	1000 µl

Table VI: Alkaline phosphatase reagents composition

Reagent No.	Name of Reagent	Composition
1.	AMP (2- amino-2- methyl-propanol buffer)	AMP– 300 mM Magnesium acetate – 2 mM Zinc sulpahte – 0.8 mM Chelator – 0.8 mM
2.	p NPP	p NPP -10mM Stabilizer – q.s.

University of Rajasthan, Jaipur (Rajasthan). The voucher of specimen samples of the plant were kept in the department for future reference. After identification the plant were cleaned well with water and dried in a shadow place. After complete drying, the fruit peel were powdered and were extracted by using soxhlet apparatus with petroleum ether, ethyl acetate and alcohol as solvents for extraction. Solvents elimination under reduced pressure afford a solid residue (yield %). Phytochemical screening gave positive tests for 17lavonoids, terpenoids, alkaloids and glycosides.[13-14]

Drug administration: Various extracts (Petroleum ether, Ethyl acetate, Alcohol) of *Annona squamosa* was suspended in distilled water and administered orally through ingastric tube at dose of 250 mg/kg body weight. The administration of the herbal extracts and standard drugs were carried out every day for 21 days. Blood samples were collected through the tail vein just prior to and on days 0,7,14 and 21 after the drug administration.

Biochemical analysis: Antidiabetic activity: Experimental design: Evaluation of antidiabetic effect of test plant extracts was done on six groups of rats by randomly selecting six rats for each group. The groups are as following.

Induction of diabetes in rats: Rats were made diabetic by single administration of streptozotocin (50 mg/kg) dissolved in 0.1M citrate buffer, pH 4.5 was intraperitoneal injected to over night fasted rats. The blood samples were collected from tail vein using capillary tubes. The blood glucose level was measured and the rats were having blood glucose level more than 200 mg/dl were considered as diabetic and used for the study.[15,16,17]

Recording of body weight: The change body weight was recorded during the study period. Body weight was measured before and after the streptozotocin administration on the 0, 7th, 14th and 21st study days during the treatment in normal control, diabetic control, standard glibenclamide, pet. ether, ethyl acetate and alcoholic extracts.[18]

Collection of blood sample: Blood samples for estimation of blood glucose was collected from each animal from the tip of the tail under mild ether as an anesthesia on 0th day (before treatment) and 7th, 14th, 21st days (during treatment). The blood samples for measuring lipids profile, liver functions tests were collected on 21st day from each animal by retro orbital route in Eppendroff's test tubes and serum was separated by centrifuge at 3000 rpm.[16,17]

Estimation of blood glucose level: Blood sugar estimation was done by using a glucometer (Accu-check® sensor, Roche Diagnostics GmbH, Mannheim) and strips. Blood glucose level was measured on the 0th, 7th, 14th and 21st study days during the treatment in normal control, diabetic mice standard glibenclamide, pet ether, alcoholic and aqueous extracts.[19,20]

Estimation of Lipid Profile[21,22,23]: Lipid profile (Total cholesterol, Triglyceride, LDL, HDL and VLDL) were estimated by using Star 21 bio auto analyser (E114947) at 505 nm by standard kits (Span diagnostics Ltd. India).

Total cholesterol: Principle: Cholesterol esters are hydrolyzed by cholesterol esterase (CE) to give free cholesterol and fatty acids. In subsequent reaction cholesterol oxidase (CHOD) oxidises the 3-OH group of free cholesterol to liberate cholest-4-en-3- one and hydrogen peroxide. In presence of peroxidase (POD), hydrogen peroxide coupies with 4-aminoantipyrine (4-AAP) and phenol to produce red quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is proportional to amount of total cholesterol concentration in the sample. Serum and other reagents were mixed well as per the following and incubated at room temperature for 30 Minutes.

$$\text{Cholesterol ester} \xrightarrow{\text{CE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Chol-st} - 4 - \text{en} - 3 - \text{one} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + 4 - \text{AAP} \xrightarrow{\text{POD}} \text{Quinoneimine} + H_2O$$

Triglycerides: Serum and other reagents were mixed well as per the following and incubated at room temperature for 30 Minutes

HDL Cholesterol: Principle: Direct HDL method: HDL is measured directly in serum: Specific reagent react with HDL-C fraction, followed by enzymatic reaction with Cholesteryl esterase(CE), Cholesterol oxidase (CO), Peroxidase(POD) with a chromogenic coupler leading to colour formation, simultaneously non HDL-C lipoprotein form colourless water soluble compound with other group of specific reagents.

$$\text{HDL Cholesterol esterase} + H_2O \xrightarrow{\text{CE}} \text{HDL unesterified cholesterol} + \text{Fatty acid}$$

$$\text{HDL} - \text{unesterified cholesterol} + O_2 \xrightarrow{\text{CO}} \text{Cholesten} - 3 - \text{One} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + 4 - \text{AAP} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + H_2O$$

Serum and other reagents were mixed well as per the

Table 1: Effect of extracts of *Annona squamosa* on body weight

Groups	Body weight (gm)				
	Before STZ	0th	7th	14th	21st
Control	161.2±5.963	160.1±5.833	160.8±5.961	161.7±6.382	162.4±7.729
Diabetic control	164.3±7.657	164.2±7.158	162.8±6.725*	160.8±5.725*	158.4±5.329*
Glibenclamide	153.9±7.232	153.2±7.441*	154.6±6.146*	155.2±5.689*	156.8±5.146*
Pet. ether extract	159.9±6.313	158.3±8.369*	159.6±8.382	160.3±7.462*	160.8±7.362*
Eth.acetate extract	160.7±5.253	159.3±8.408*	160.5±7.441*	161.3±6.158*	162.1±5.284*
Alcoholic extract	158.8±6.214	158.1±6.146*	159.6±6.110*	160.7±5.764*	162.5±5.689*

Data are expressed as mean ï SEM; n=6 animals in each group; P<0.05: Statistically significant from diabetic control; Statistical analysis was carried out using Graph Pad PRISM software (version 4.03); One way ANOVA was used, followed by Bonferroni multiple comparison tests.*

Table 2: Effect of *Annona squamosa* on blood glucose level

Groups	Blood glucose (mg/dl)			
	0 day	7th day	14th day	21th day
Control	86.2±1.713	86.5±1.317	86.8±1.610	86.5±0.992
Diabetic control	288.6±2.376	292.7±4.502	301.8±3.120	318.6±4.471
Glibenclamide	270.6±1.783*	168.3±1.332*	152.8 ±1.878*	141.6±1.113*
Pet. ether extract	289.5±2.325*	286.5±2.596	282.7±1.659*	273.7±1.436*
Eth.acetate extract	288.5±3.375	255.2±2.298*	221.7±1.573*	213.7±1.174*
Alcoholic extract	284.5±2.825*	245.1±1.461*	207.1±1.059*	197.2±1.485*

*The results were expressed as mean ï SEM; n=6 animals in each group; * P<0.05: Statistically significant from diabetic control; Statistical analysis was carried out using Graph Pad PRISM software (version 4.03); One way ANOVA was used, followed by Bonferroni multiple comparison tests; Diabetic control was compared with control mice. Diabetic + Glibenclamide, diabetic + Pet ether, diabetic + Eth.acetae extract and diabetic + alcoholic extract were compared with diabetic control.*

Table 3: Effect of *Annona squamosa* on lipid profile

Drug treatment	Total cholesterol (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
Control	94.79±1.376	62.72±1.011	47.62±1.182	38.66±0.874	11.48±0.672
Diabetic control	145.03±1.979	93.25±2.191	82.47±1.252	23.72±1.439	19.47±0.479
Glibenclamide	98.21±1.928*	65.91±1.285*	42.31±1.505*	39.13±0.871*	12.24±0.187*
Pet. Ether extract	127.38±1.414*	85.57±1.667*	71.35±1.737*	25.14±1.798*	21.78±0.981*
Eth.acetate extract	116.34±1.867*	81.86±2.751*	67.33±2.917*	27.37±1.261*	18.38±0.798*
Alcoholic extract	108.83±1.651*	73.28±1.283*	53.56±1.384*	31.23±1.617*	16.38±0.821*

*Data are expressed as mean ï SEM; n=6 animals in each group; * P<0.05: Statistically significant from diabetic control; Statistical analysis was carried out using Graph Pad PRISM software (version 4.03). One way ANOVA was used, followed by Bonferroni multiple comparison tests.*

following and incubated at room temperature for 30 minutes

LDL cholesterol

Friedewalds equation

$$\text{LDL cholesterol} = \text{Total cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL cholesterol}$$

VLDL cholesterol: Calculation

VLDL cholesterol was Calculated by using Friedewalds equation

$$\text{VLDL} = \frac{\text{Triglycerides}}{5}$$

Estimation Of Liver Physiological Profile [24,25]

Liver function parameters (ALP, SGOT, SGPT & Total bilirubin) estimations were carried out by Star 21 bio auto

Table 4: Effect of *Annona squamosa* on liver function parameters

Groups	ALP (IU/L)	SGOT (IU/L)	SGPT (IU/L)	Bilirubin (IU/L)
Control	156.08±3.532	65.6±2.356	51.52±2.651	0.72±0.011
Diabetic control	267.81±3.114	125.51±1.744	105.41±2.312	2.12±0.094
Glibenclamide	172.18±2.063*	58.32±1.511*	63.20±2.451*	0.65±0.515*
Pet. ether extract	235.17±2.702*	103.82±2.642*	88.74±1.424*	2.08±0.951*
Eth.acetate extract	204.44±2.463*	96.35±2.139*	77.56±2.384*	1.74±0.068*
Alcoholic extract	198.37±1.432*	89.47±2.595*	69.87±2.568*	1.48±0.022*

*Data are expressed as mean ï SEM; n=6 animals in each group; * P<0.05: Statistically significant from diabetic control; Statistical analysis was carried out using Graph Pad PRISM software (version 4.03). One way ANOVA was used, followed by Bonferroni multiple comparison tests.*

analyser (E114947), using standard kits (Span diagnostics Ltd. India)

Alkaline phosphatase (ALP): Principle: Estimation of alkaline phosphate (ALP) at pH 10.3, involves hydrolysis of colourless P-nitrophenyl phosphate by alkaline phosphatase to give p-nitrophenol, which gives yellow color in alkaline solution. The increase in absorbance due to its formation is directly proportional to alkaline phosphate (ALP) activity. Change in absorbance due to yellow colour formation is measured kinetically at 405 nm.

$$p-\text{Nitrophenyl phosphate} + H_2O \xrightarrow{ALP} p-\text{Nitrophenol} + \text{Phosphate}$$

Working reagent was prepared by reconstituting one vial of reagent 2, with 1.2 ml reagent 1, dissolve properly by gentle swirling. 20 µl serum volume was added in 1 ml working ALP reagent, at pH 10.3 and was then kept 30 second at room temp.

Serum glutamic pyruvate transaminase (SGPT): Principle: The alanine aminotransferase (ALT), formerly called glutamic pyruvic transminase (GTP), which catalyze the conversion of α – keto acids into amino acids by transfer of amino groups and the pyruvate formed in the reaction is converted to lactate by lactate dehydrogenase (LDH) with simultaneous oxidation of NADH to NAD and the decrease in absorbance at 340 nm. Optimized UV-test according to International federation of clinical chemical chemistry and Laboratory medicine (IFCC).

$$L-\text{alanine} + \alpha-\text{ketoglutarate} \xrightarrow{ALT} L-\text{glutamate} + \text{pyruvate}$$

$$\text{Pyruvate} + NADH + H^+ \xrightarrow{LDH} L-\text{lactate} + NAD^+$$

Procedure: 0.1 ml serum volume was added in 1 ml working reagent and was then kept for 1 minute at room temp.1 ml of working reagent was prepared by mixing 800 µl of reagent 1 (Tris buffer pH 7.5, L- alanine, LDH) with 0.2 ml reagent 2 (α – ketoglutarate , NADH). Activity was measured at 340 nm on U.V. using autoanalyzer.

Serum glutamic oxaloacetate transaminase (SGOT): Principle: The aspartate aminotransferase (AST), formaly called glutamic oxalacetic transaminase (GOT), which catalyze the conversion of α – keto acids into amino acids by transfer of amino groups and the oxaloacetate formed in the reaction is converted to malate by malate dehydrogenase (MDH) with simultaneous oxidation of NADH to NAD and the decrease in absorbance at 340 nm. Optimized UV-test according to International federation of clinical chemical chemistry and laboratory medicine (IFCC). Activity was measured at 340 nm on U.V. using autoanalyzer.

$$L-\text{alanine} + \alpha-\text{ketoglutarate} \xrightarrow{AST} L-\text{glutamate} + \text{pyruvate}$$

$$\text{Pyruvate} + NADH + H^+ \xrightarrow{MDH} L-\text{lactate} + NAD^+$$

Procedure: 0.1 ml serum volume was added in 1 ml working reagent and was then kept 1 minute at room temp. 1 ml of working reagent was prepared by mixing 0.8 ml of reagent 1 (Tris buffer pH 7.5, L- aspartate, MDH) with 200 µl reagent 2 (α – ketoglutarate , NADH).

Total bilirubin: Principle: Estimation of total bilirubin (TB) involved the reaction of bilirubin with diazotized sulfanilic acid to form an azo compound, the color of which is measured at 546 nm.

Procedure: 0.1 ml serum volumes were added in 0.1 ml working reagent at room temperature. Working reagent prepared by mixing reagent 1 (sulfanilic acid solution, HCl) and reagent 2 (sodium nitrite solution) in the ratio of 4:1 to make a diazo solution.

STATISTICAL ANALYSIS

The results were expressed as mean ± SEM; n=6 animals in each group; * P<0.05: Statistically significant from diabetic control; Statistical analysis was carried out using Graph Pad PRISM software (version 4.03). One way ANOVA was used, followed by Bonferroni multiple comparison tests; Diabetic control was compared with control rats. Diabetic + Glibenclamide, diabetic + Pet ether extract, diabetic + Ethyl acetate extract and diabetic + alcoholic extract were compared with diabetic control.

RESULTS

The results of body weight, blood glucose level, lipid profile and liver function parameters of normal control

group, diabetic control group, standard group (Glibenclamide 500 µg/kg) and three different extracts (Petroleum ether, Ethyl acetate and Alcohol) of herbal drug *Annona squamosa* were summarized in table no.1,2,3,4.

Effect of extracts on body weight: Table-1 shows that a significant decrease was observed in the body weight of diabetic rats compared with control rats. Treatment with extracts of fruit peel of *Annona squamosa* and glibenclamide, the body weight gain was improved but the effect was more pronounced in alcoholic extracts of *Annona squamosa* treated rats than glibenclamide on 14, 21st day of study.

Estimation of blood glucose level: Table-2 shows that treatment with oral glibenclamide & various extracts of fruit peel of *Annona squamosa* diminished blood glucose level on day 0, 7th, 14th & 21. The untreated diabetic control rat group showed increase in blood glucose level throughout the entire study period. Initially blood glucose level of untreated diabetic control group was 288.6±2.376 and after 21 days of trial period the blood glucose level was increased to 318.6±4.471. For trial drugs *Annona squamosa* (250 mg/kg) blood glucose were studied in three different groups of animals. All the three groups showed a significant decrease of blood glucose level on streptozotocin induced diabetic rats when compared to control goup. The initial readings of blood glucose level of Petroleum ether, Ethyl acetate and Alcoholic extract were 289.5±2.325, 288.5±3.375 and 284.5±2.825 respectively. After the trial period, there was marked reduction in blood glucose levels 273.7±1.436, 213.7±1.174 and 197.2±1.485 in 21 days. However alcoholic extract of *Annona squamosa* has shows maximum effect than petroleum ether and ethyl acetate. In standard group initial blood glucose was 270.6±1.783 and the post test was 141.6±1.113 which showed that the standard drug produced maximum hypoglycemic effect and the statistical analysis was extremely significant and slightly higher than that of trial drug group.

Estimation of Lipid Profile of *Annona Squamosa:* Table-3 shows the levels of Total Cholesterol(TC), Triglycerides(TG's), Low Density Lipids(LDL), High Density Lipids(HDL) and Very Low Density Lipids(VLDL) levels in liver of control and experimental rats. The results showed that increased levels of TC, TG's, LDL and VLDL in diabetic rats when compared with normal rats. In rats treated with different extracts of *Annona squamosa* and Glibenclamide there was a significant decrease in content of TC, TG's, LDL and VLDL levels and significantly increase in HDL levels when compared with diabetic control rats.

Estimation of Liver Physiological Profile of *Annona Squamosa:* Table-4 shows the levels of ALP, SGOT, SGPT and Bilirubin in liver of control and experimental rats. The results showed that increased levels of ALP, SGOT, SGPT and Bilirubin in diabetic rats when compared with normal rats. In rats treated with different extracts of *Annona squamosa* and Glibenclamide there was a significant decrease in content of ALP, SGOT, SGPT and Bilirubin levels when compared with diabetic control rats.

DISCUSSION

Diabetes mellitus is one of the most common chronic disease and is associated with hyperglcaemia, hyperlipidemia and co-morbidites such as obesity, hypertension. Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes.[26] Streptozotocin selectively destroys pancreatic insulin secreting β-cells causing diabetes close to type-2 diabetes of humans.[27] Streptozotocin induces a wide variety of animals species by damaging the insulin secreting pancreatic β-cells, resulting in a disease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues.[28] Since streptozotocin is known to destroy pancreatic β-cells, the present findings appear to be in consonance with the earlier suggestion that sulfonyl urea have extra pancreatic antihyperglycemic mechanism of action secondary to their insulin secreting effect and attendant glucose uptake into and utilization by the tissues.[27] Apart from regulation of carbohydrates metabolism, insulin also plays an important role in metabolism of lipids. Insulin is potent inhibitor of lipolysis. Since it inhibits the activity of the hormone sensitive lipases in adipose tissue and suppresses the release of free fatty acids.[28] During diabetes, enhanced activity of this enzyme increases lipolysis and release more free fatty acids, into the circulation.[29] It is well known that in uncontrolled type-2 diabetes mellitus, there will be an increase in TC, LDL, VLDL cholesterol and TG's with decrease in HDL cholesterol which contribute to the coronary artery disease

Based on the results, it can be observed that there was an extremely significant reduction in blood glucose level by herbal drug *Annona squamosa* in streptozotocin induced diabetic rats. The maximum result obtained from alcoholic extract of *Annona squamosa*. The declined trend was observed at constant levels. The antidiabetic activity of *Annona squamosa* may be due to increased release of insulin from β-cells of pancreas or it may potentiate the effect of insulin. Treatment of *Annona squamosa* in diabetic rats also showed the highly significant weight gain property which favours the beneficial effect of *Annona squamosa* in treating diabetic patients.[30,31]

The various extracts (Petroleum ether, Ethyl acetate and Alcohol) of *Annona squamosa* brought down the elevated levels of TC, LDL, VLDL cholesterol and TG's in diabetic animals to nearly normal level. There was increase in HDL cholesterol also, was a desirable feature.

The various extracts (Petroleum ether, Ethyl acetate and Alcohol) of *Annona squamosa* brought down the elevated levels of liver function parameters such as ALP, SGPT, SGOT and Bilirubin in diabetic animals to nearly normal level also. The elevated levels of SGPT, SGOT reduced by the treatment of *Annona squamosa* which might be a consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by STZ. Increased in serum ALP level might be due to increased synthesis in presence of billary pressure. The alcoholic extract *of Annona squamosa* had shown significant reduction in serum ALP and bilirubin, indicated an improvement in the secretory mechanism.

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