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Biochemical approach for the Evaluation of Anti-Diabetic Potential of Leaf Extract of *Annona squamosa* in Streptozotocin Induced Diabetic Mice.

Vinay Bhushan Kumar[1], Sushila Kumari[2], Sanjay Kumar Sharma[3], Shashi Bhushan Lal[3], Kumari Savita[4]

[1]*TPS College, Patliputra University, Patna, India*
[2]*Microbial and Molecular genetics Laboratory, Patna University, Patna, India*
[3]*Department of Zoology, Patna University, Patna, India*
[4]*Department of Biochemistry, Patna University, Patna, India*

Abstract
Background: *Management of the diabetes is a greatest challenge before the generation, as it is engulfing 10% of the total healthcare budget alone. Treatment with the synthetic hypoglycemic approach presents severe side effects with piles up the diabetic complications. Treatment of diabetes with the aid of herbal is gaining much attention. Present study evaluates the Ethanolic extract of the Annona squamosa for reducing hyperglycemia along with other biochemical alteration.*
Materials and method: *Study included thirty male rats divided into five groups, Group I Normal control, Group II-Diabetic control, Group III- A. squamosa extract (150 mg/kg) (DT_{150}), Group IV- A. squamosa extract (250 mg/kg) (DT_{250}), and Group V- Rosiglitazone (2 mg/kg) (DT_{RGZ}). The rats in the groups were evaluated for fifteen days. Blood sample and liver tissues were collected for further biochemical analysis. Statistical analysis was done using MATLAB software and data were represented as Mean±SEM.*
Results: *Results of the desired parameters were found significant. The parameters considered for the study were, body weight, fasting insulin, blood glucose, lipid profile, ALT, AST, urea creatinine in blood sample and Glucokinase, glycogen, Glucose- phosphatase in hepatic tissues. Results obtained were compared with that of Rosiglitazone for the efficacy and potential evaluation of herbal with synthetic one.*
Conclusion: *According to results obtained it could be concluded that the herbal extract of Annona squamosa could be better alternative against the synthetic drugs for reducing the diabetic complications in a natural way. However, translational research is required on human with large sample size because the route of administration and physiology of human differs with that of the mice.*
Key words: *Annona squamosa, Hyperglycemia, ALT, AST, Lipid profile, Rosiglitazone*

I. Introduction

India has emerged as one of the major epicenters of the global diabetes mellitus pandemic. Rapid development in socioeconomic status and demographic changes has led the Indian population with increased susceptibility for explosive prevalence of diabetes mellitus in past four decades [1]. Statistical analysis of diabetes represents that there are about 463 million adults having age between 20-79 years who are suffering from this disease which is projected to increase by 700 million by 2050. Its intensity is increasing in low and middle-income countries which are 3 in 4 (79%) are diabetes sufferers. it is estimated that 1 in 5 people (136 million) of age above 65 years are having diabetes and 232 million are undiagnosed. Diabetes caused 760 billion USD expenditure in 2019 which is 10% of the total health expenditure. More than 20 million live births (1 in 6 births) are affected by hyperglycemia and out of this 84% developed gestational diabetes mellitus. 764 million candidates are at increased risk of developing diabetes globally[2].

Insulin resistance (IR) has emerged as a major pathophysiological factor in the development and progression of DM which evidences the individual's susceptibility at the early for type 2 Diabetes Mellitus (T2DM) earlier. Therefore, screening of IR through homeostasis model assessment of IR (HOMA-IR) is a key indicator for the earlier prevention of type 2 Diabetes Mellitus[3]. Diabetes complications are responsible for significant morbidity and mortality. The chronic complications of diabetes are broadly divided into microvascular and macrovascular,of which microvascular have much higher prevalence than other one[4]. Microvascular complication includes neuropathy, nephropathy, and retinopathy, while macrovascular complications consist of cardiovascular disease, stroke, and peripheral artery disease [5].

Naturally obtaining phytoactive compounds and herbs are very important because they found to be effective against several diseases. There are several oral hypoglycemic agents available in the market but in the long term may lead to a high risk of secondary failure rate[6]. We observed that owing the potential of natural products and herbs different research groups are searching for the potent natural antidiabetic agents with minimal side effects. Recent research suggests decline in the number of new molecules discovery due to failing in clinical trial because of toxicity and its associated side effects thus natural products and herbals has emerged as the alternative. Presently, some of the natural products and herbs like coixol, andrographolide, *Tinospora cordifolia*, polypeptide p, charantin, Annona squamosa and Nigella are being explored for their potential to be used successfully for the management of type 2 Diabetes[6]. Present study deals with the anti-diabetic properties of the plants Annona squamosa, commonly known as the custard apple tree is a native of West Indies. But the cultivation is present throughout throughout the regions of tropics and India, because of its edible nature. It belongs to Class Magnoliopsida, and family of Annonaceae.

Previous literatures highlights about its beneficial effect for the treatment of various ailments most likely, The leaves served as a purgative, Decoction of the leaves and/or root is taken in cases of dysentery, diabetes, digestive problem, treat colds, and rheumatic pain etc. In the study it was found that Oral administration of *A. squamosa* (300 mg/kg) aqueous extract to diabetic rats for 30 days significantly reduced blood glucose, urea, uric acid and creatinine, increased the activities of insulin, C-peptide, albumin, albumin/globulin ratio and restored all marker enzymes to near control levels [7]. There is an evidence for reducing oxidative stress like by maintaining of the oxidative enzymes like catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione reductase (GR) and glutathione-s-transferase (GST) and decrease in malondialdehyde levels present in various tissues and the extract extract also improved the lipid profile[8]. Translational research using the desired herbal showed important outcome like combination of *Annona squamosa* along with Glipizide may be helpful in dose reduction of Glipizide up to 50%, reducing the risk of the onset of insulin therapy[9]. In an another research it was evidenced that the hexane extract of *A. squamosa* (100 and 400 mg/kg body weight) dose significantly increased insulin level as compared to Glimepiride (1 mg/kg) and also inhibited alpha-glucosidase activity when compared with Acarbose (10 mg/kg) in streptozotocin induced diabetic rats[10]. Previous literatures also finds that the extract of *Annona squamosa* houses varieties of phytochemicals of which annonaceous acetogenins (ACGs), diterpenes (DITs), alkaloids (ALKs) and cyclopeptides (CPs) as the main constituents and Until 2016, 33 DITs, 19 ALKs, 88 ACGs and 13 CPs from this species were reported[11]. For the induction of Diabetes, streptozotocin was utilized which is a glucosamine-nitrosourea compound and it causes toxicity to cells by damaging DNA, and by other mechanism like increasing oxidative stress causing damage to β-cell along with other histological alterations. Streptozotocin is a glucose analogue hence it is allowed to transported into the cell through glucose transport protein GLUT2, and β-cells have relatively high levels of GLUT-2 transporters and after reaching to β-cells streptozotocin causes toxicity to these cells and hence cell death occurs in mass [12].

II. Materials And Method

Experiment was designed according to need of the topic that is effect of *Annona squamosa* on the diabetic mice.

Albino mice: The albino mouse is an excellent model therefore; all mice used in this study were in the albino genetic background. Adult albino mice weighing around 17-20 gram with 6.5 ± 0.5 cm length were selected for experiments. Optimum growth temperature ranging in between 22-25° C. The relative humidity of the room was maintained between 50 and 55 percent. Twelve hours of lighting (with light intensity between 350-400 lux) and twelve hours of darkness was provided in the rooms for optimal growth and reproduction.

Grouping of the mice

Group I	Normal control
Group II	Diabetic control
Group III	*A. squamosa* extract (150 mg/kg) (DT_{150})
Group IV	*A. squamosa* extract (250 mg/kg) (DT_{250})
Group V	Rosiglitazone (2 mg/kg) (DT_{RGZ})

Plant materials: leaf of *Annona squamosa Linn*.

Preparation of Herbal extract: The powder of *Annona* (weighted 940gms) after the grinding was kept in the separate perculator (made up of glass) and filled up with about 3 liters commercial alcohol (95% ethanol and 5% water) more than 1" or $1^1/_2$" than powder and left it 24 hours. After 24 hours, the whole dissolved solution (all constituent of *Annona*) is drained out in a 5000 ml conical flask. About 50 ml. dissolved solution was taken in 300 ml Round bottle flask from the 5000ml conical flask and evaporated under reduced pressure and low temperature (60° C) in Rotavapour (Popular India). In Rotavapour, the commercial alcohol was vaporized and plants extract remains in the round bottle flask. The remaining plant extract is collected from the scaptula from

round bottle flask and kept in the plastic jar. Again, the commercial alcohol was filled up in the perculator and left it 24 hours.

The above process is repeated again 4 times for complete extraction of plant extract.

Diabetogenic material and its preparation: Streptozotocin is a Diabetogenic material in the present research work, obtained from merk and it was dissolved in 1 m Molar citrate buffer at pH 4.5. Mice were kept to a 12-hour fast, and it was induced by injection of freshly prepared working STZ solution (40mg/kg body weight) inttaperritonealy. The Diabetic rats were administered with ethanolic extract of *Annona squamosa* for the mentioned period and subjected to various biochemical and histological analysis. For Biochemical analysis blood samaples were collected and for other parameters like glucokinase, glycogen, glc-6-phosphatase liver tissue were isolated.

Biochemical Estimation

The desired Biochemical parameters were accessed to monitor the metabolic activity of the mice in the respective groups.

Fasting Plasma Glucose by GOD POD method [13], serum Cholesterol CHOD POD , triglyceride using GPO method, HDL by Phosphotungestic method[14]. Serum LDL and VLDL were calculated using Frieldwald formula[15], serum creatinine by alkaline picrate method[16], Serum urea by Nitroprussic method, Alanine aminotransferase (ALT) Reitman and Frankel method and Aspartate aminitransferases (AST) Modified IFCC method [17] and HOMA-IR [18].

Histopathology estimation

The liver tissues were homogenized for the evaluation of hepatic glycogen level[19], G6Pase [20] and Glucokinase activities[21]

Statistical Analysis

Data were expressed as the mean ± S.E.M. For statistical analysis of the data, group means were compared by one-way ANOVA with *Post Hoc* analysis. The Tukey–Karmer *Post Hoc* test was applied to identify significance among groups. Graphs are plotted using MATLAB version 7.8.0, Massachusetts: The Mathworks Inc. 2009.

III. Results

The leaf extract of *Annona squamosa* has been reported to be effective in the alleviation of diabetes through its antioxidant and insulin-potentiating activities[22]. This study evaluates systematically, the beneficial effects of methanolic extract of .leaf of *Annona squamosa* in streptozotocin (STZ) induced diabetic albino mice.

Effect of *Annona squamosa* extract on body weight

The diabetic control (DC) mice presented significantly lower body weight ($p<0.001$) when compared with the normal control (NC) mice (Table 1). A significant body weight gains were observed in the treated groups of diabetic mice (DT_{150} and DT_{250}) as compared to the DC ones (Table I). The DT_{150} and DT_{250} group showed an increase of 18% and 25% in body weight respectively after 15 days of treatment. Contrary to this, DT_{RGZ} group mice showed an increase of 30% in body weight after 15 days of treatment

Table no I. Shows Fluctuation of weight in the mentioned duration

	Groups	Day 0	Day 7	Day 15
	Normal control (NC)	17.90 ± 2.74	19.84 ± 2.45	22.78 ± 2.21
Diabetic mice	**Diabetic control (DC)**	11.72±1.04	09.50±0.85	9.31±1.31
	***A. squamosa* extract (150 mg/kg) (DT_{150})**	11.71±2.02*	12.0.8±1.65*	13.78±1.51*
	***A. squamosa* extract (250 mg/kg) (DT_{250})**	10.88±1.61*	13.72±1.27*	14.74±2.265*
	Rosiglitazone (2 mg/kg) (DT_{RGZ})	10.60 ± 2.76*	13.82 ± 2.89*	15.02 ± 1.04*

** $p<0.05$ as compared with normal control. ** $p < 0.01$ as compared with diabetic control.*

Effect of *Annona squamosa* extract on blood glucose level

As expected, the DC mice showed significantly ($p<0.001$) higher level of glucose (+279%), when compared with their normal control counterparts (Table II). Diabetic mice of both of the groups (DT_{150} and DT_{250}) showed a reduction in glucose levels, however DT_{250} was more effective. DT_{RGZ} group mice showed nearly 67% decrease in glucose level after 4-weeks of treatment program as compared to the diabetic subjects.

Table no II. Shows concentration fasting plasma glucose for the mentioned period in the groups

GROUPS	Blood glucose levels (mmol/l)				
	Pretreatment (week)	Post-treatment (week)			
	0	1	2	3	4

Normal control	3.98 ±0.11**	4.07±0.18**	4.06±0.21**	4.05±0.14**	3.99±0.16**
Diabetic control	14.94±1.53*	14.91±1.43*	14.78±1.54*	14.99±1.47*	14.94±1.49*
Rosiglitazone (2 mg/kg)	15.01±1.44	9.88±1.42**	5.56±1.24**	4.95±1.31**	4.96±0.94**
***Annona* extract (250mg/kg)**	14.60±1.50	11.83±1.30**	9.68±1.23**	8.26±1.73**	8.17±1.21**
***Annona* extract (150mg/kg)**	14.90±1.48	13.09±1.18*	10.65±2.01**	9.68±1.24**	9.24±1.78**

** p<0.05 as compared with normal control. ** p < 0.01 as compared with diabetic control.*

FINS (Fasting Insulin) levels

The HOMA-IR of the diabetic control were much higher than those of the normal control group (p<0.01). When the mice had been administered with vehicle or experimental compounds, respectively, for 28 days, the FINS levels of *Annona* extract at 150 mg/kg body wt/day and 250 mg/kg body wt/day doses and rosiglitazone groups were significantly lower as compared to diabetic control (p<0.01), as were the FBG levels (p<0.01). Therefore, the *Annona squamosa* extract at 150mg/kg body wt/day and 250 mg/kg body wt/day treated groups had lower HOMA-IR as compared to diabetic control (p<0.01)(Table III).

Table no III. Represents the concentration of fasting insulin and calculated HOMA-IR in the groups

Groups	**FINS(mIU/L)**	**HOMA-IR**
Normal control	23.21±2.11**	4.93±1.11**
Diabetic control	34.46±3.15*	22. ± ±2.69*
Rosiglitazone (2 mg/kg)	24.04±2.41**	5.11±1.21**
***Annona* extract (250mg/kg)**	25.38±1.58**	9.82±1.66 **
***Annona* extract (150mg/kg)**	26.84±1.74**	10.96±1.84**

** p<0.05 as compared with normal control. ** p < 0.01 as compared with diabetic control.*

Effect of *Annona squamosa* extract on lipid profile

When compared with normal control, the diabetic mice had higher total cholesterol (TC) (+136%; $p<0.001$) and TGs (+71%; $p<0.001$) values. Diabetic mice treated with lower dose of *Annona squamosa* extract (DT_{150}) showed significantly lower values of serum TC (-27%; $p<0.001$) and TGs (-56.6%; $p<0.001$), when compared with the DC counterparts (Table). The DT_{250} treatment showed superior lowering effects compared with the DC counterparts as well as DT_{150} group mice by (-35.7%; $p<0.001$) on serum TC levels and (-49%; $p<0.001$) on TGs levels (Table). Contrarily, treatment with rosiglitazone (DT_{RGZ}) showed (-34.2%; $p<0.001$) on TC levels and (-10.6%; $p<0.001$) on TGs levels compared with diabetic control mice (Table IV).

Relative to normal control, the diabetic mice had higher value of low density lipoprotein (LDL) (+257%; $p<0.001$) while diminished value of high density lipoprotein (HDL) (-55%; $p<0.001$). Diabetic mice treated with lower dose of *Annona squamosa* extract (DT_{150}) showed significantly lower values of serum LDL (-52%; $p<0.001$) and higher value of HDL (-49.4%; $p<0.001$), when compared with the DC counterparts (Table). All over again, the DT_{250} treatment showed even better lowering effects on LDL (-54.7%; $p<0.001$) compared with the DC counterparts as well as DT_{150} group mice and improved level of HDL (+52.9%; $p<0.001$) (Table). In contrast, treatment with rosiglitazone (DT_{RGZ}) showed a considerable diminished level of LDL (-57%; $p<0.001$) while improved level of HDL (+55%; $p<0.001$) compared with diabetic control mice (Table IV).

Table no IV. Represents lipid profile status in extract treated groups, Normal control, and Diabetic control after 15 days

Groups	**TC(mmol/L)**	**TG(mmol/L)**	**HDL(mmol/L)**	**HDL/TC (%)**	**LDL(mmol/L)**
Normal control (NC)	4.15±0.86**	1.14±0.09**	2.86±0.29**	68.91±4.66**	0.27±0.04**
Diabetic control (DC)	9.84±1.56*	1.96±0.29*	1.31±0.58*	13.31±1.97*	0.96±0.16*
***A. squamosa* extract (150 mg/kg) (DT_{150})**	7.23±0.44**	0.87±0.08**	2.54±0.36	35.13±3.37**	0.47±0.06**
***A. squamosa* extract (250 mg/kg) (DT_{250})**	6.42±0.64**	0.98±0.17**	2.78±0.46**	43.39±4.26**	0.44±0.07**
Rosiglitazone (2 mg/kg) (DT_{RGZ})	6.58±1.35**	1.77±0.17**	2.90±0.55**	44.07±5.56**	0.41±0.09**

** p<0.05 as compared with normal control. ** p < 0.01 as compared with diabetic control.*
TC = Total Cholesterol; TG = Triglycerides; HDL = High density lipoprotein; LDL = Low density lipoprotein

Effect of *Annona squamosa* extracts on kidney function markers

Diabetic mice have higher levels (approximately twice) of serum urea, creatinine. In DT_{RGZ} mice the parameters, serum urea and serum creatinine were reduced by 134% and 52% respectively (Table). Treatment with *Annona squamosa* extract decreases the values of Urea and creatinine in a dose dependent manner when compared to diabetic control mice. The maximum efficacious dose was found to be 250 mg/kg body weight of mice (Table V). Thus the result showed that the *Annona squamosa* extract is also as effective as Rosiglitazone in improving the kidney function.

Table no V. Represents kidney marker status in extract treated groups, Normal control, and Diabetic control after 15 days

Groups	Urea (mg/dl)	Creatinine (mg/dl)
Normal control (NC)	38.15 ± 0.44**	0.89 ± 0.038**
Diabetic control (DC)	90.45 ± 1.73*	1.48 ± 0.037*
***A. squamosa* extract (150 mg/kg) (DT_{150})**	48.22 ± 1.15**	1.24 ± 0.054**
***A. squamosa* extract (250 mg/kg) (DT_{250})**	37.79 ± 2.36**	1.06 ± 0.026**
Rosiglitazone (2 mg/kg) (DT_{RGZ})	38.57 ± 0.15	0.97 ± 0.025

** p<0.05 as compared with normal control. ** p < 0.01 as compared with diabetic control.*

Effect of *Annona squamosa* extracts on liver function markers

There was a significant elevation in transaminase activity (SGOT and SGPT) in liver in diabetic mice, when compared to normal control mice. Extract treated group showed significant recovery in both the parameters (150 and 250mg/kg body weight). The data suggest recovery in 250mg/kg body was more as compared to Rosiglitazone trteated group (p<0.01) (Table VI).

Table no VI. Represents liver markers status in extract treated groups, Normal control, and Diabetic control after 15 days.

Groups	SGPT (IU/L)	SGOT (IU/L)
Normal control (NC)	28.47 ± 0.48**	67.13 ± 1.43**
Diabetic control (DC)	63.75 ± 1.74*	112.42 ± 2.43*
A. squamosa extract (150 mg/kg) (DT150)	32.85 ± 2.94**	63.48 ± 0.75**
A. squamosa extract (250 mg/kg) (DT250)	27.74 ± 0.55*	63.87 ± 1.45*
Rosiglitazone (2 mg/kg) (DTRGZ)	30.94 ± 0.57	73.47 ± 1.76

** p<0.05 as compared with normal control. ** p < 0.01 as compared with diabetic control.*

Effect of *Annona squamosa* Glycogen content, G6Pase activity and GK activity in liver

Glycogen content was increased by 54.13% and 60.95% (p<0.01), respectively, in the 250 mg/kg body wt/day treated dose and 150 mg/kg body wt/day treated dose of *Annona squamosa* groups, and by 76.57% and 59.07% (p<0.01), respectively, as compared with diabetic control. G6Pase activity was assessed in all groups (Table VII). Compared with diabetic control, G6Pase activity was decreased by 37.50% and 51.56% respectively, in the 250 mg/kg body wt/day, 150 mg/kg body wt/day of *Annona squamosa* groups (p<0.01), as compared with diabetic control. As shown in (Table VII) GK activity was increased by 61.61 and 58.93 respectively, in the 250 mg/kg body wt/day, 150 mg/kg body wt/day dose of *Annona squamosa* groups (p<0.01), as compared with diabetic control

Table no VII. Represents Glycogen, Glucokinase and Glucose--Phosphatase status in extract treated groups, Normal control, and Diabetic control after 15 days.

Groups	Glycogen content (mg/g)	Glucose-6-phosphatase activity (mU)	Glucokinase activity (mU)
Normal control	16.85±1.4**	0.32±0.04**	3.53±0.16**
Diabetic control	11.14±1.1*	0.64±0.05*	1.12±0.09*
Rosiglitazone (2 mg/kg)	13.62±1.6**	0.41±0.06**	1.71±0.16**
***Annona* extract (150 mg/kg)**	15.17±1.5**	0.38±0.03**	1.81±0.11**
***Annona* extract (250 mg/kg)**	17.93±2.1**	0.40±0.05**	3.08±0.14**

** p<0.05 as compared with normal control. ** p < 0.01 as compared with diabetic control.*

Thus, the current results demonstrate the anti-diabetic effect of *Annona squamosa* extract. The *Annona squamosa* extract has the potential to play a role in the prevention of diabetes and its resulting complications and could promote a better health.

IV. Discussion

Diabetes has a significant impact on the health, quality of life and life expectancy of patients as well as healthcare expenditure. With increasing incidence and mortality from its associated complications, prompt and adequate glycemic control in diabetes is most important, proper management can consequently increase the life

expectancy[23]. Diabetic rat in the experiment showed characteristic loss of body weight[24], where as treatment with the herbal extract of *A. squamosa* showed improvement and significant gain in their body weight (Table I), this was probably due to control muscle waste, reduced physical discomfort and decreased stress. This result was in accordance with [25].

Type 2 Diabetes characterizes hyperglycemia which is the result of insufficient amount amount of Insulin secreted from β-cell leading to relative Insulin deficiency and increase level of glucose in plasma. Daily oral administration of the extract for 28 days produced a gradual but sustained reduction in blood glucose and increased insulin levels in diabetic treated mice. Glucose lowering effect of *Annona* extract might be due to stimulation of surviving β-cells of Islets of Langerhans leading to the increased pancreatic secretion of insulin from β-cells and hence decreased glucose concentration after treatment (Table II). The present investigation also registers a significant ($p<0.01$) decrease in Fasting insulin (FINS) and HOMA-IR level (Table III) and the results were in accordance with the previous findings [26, 27] .

In present study, abrupt increase in serum lipid level in diabetic mice especially in LDL, TG and TC level, while decrease in HDL/TC level up to 33.21% was noticed. Decreased clearance in T2D may lead to increased LDL concentration[28] whereas, decreased HDL concentration in T2D may be related to the activity of adipose tissue lipoprotein Lipase (LPL), because LPL deficiency may be a factor responsible for altered distribution of HDL particle in untreated T2D[29] such alteration in lipoprotein can lead to cardiovascular complications. Following the administration of *Annona* extract, there was significant ($p<0.01$) reduction in LDL, TG, and TC concentration was achieved as compared to normal and diabetic control ($p<0.05$ and $p<0.01$), overall *Annona* extract lowered TC, TG and LDL level and effects were more marked compared to Rosiglitazone (Table IV). Thus it is reasonable to conclude that *Annona* extract could modulate lipid abnormalities. The hypolipidemic effect of *Annona* extract may be due to activation of lipoprotein lipase (LPL) and stimulation of β-cell to secrete sufficient insulin to clear Triglycerides from plasma[30]

In the study, STZ induced diabetic mice showed a marked increase in urea and creatinine level as compared to normal control. Excess urea and creatinine is marker of defective kidney function which may be due to effect of hyperglycemia and increased oxidative stress. After the administration of *A. squamosa* extract, there was significant decrease in serum urea and creatinine level indicates protective effect of *Annona* extract on kidney. The *Annona* extract was effective in 250 mg/kg body wt/day dose (Table V). The extract of *Annona* helps to preserve kidney function towards normal by ameliorating histopathological changes through reduction of, inflammation, fibrosis, and apoptosis in diabetic mice. The present results corroborate with the previous findings[31].

Liver is the central organ for the metabolism, in diabetic state it is represented with altered hepatic enzymes concentration. Present research finds increased concentration of ALT and AST which suggests histoarchitectral abnormalities which might have occurred due to the oxidative stress and tissue ischemia pertaining to leaking of this enzyme in to the blood pool. After the extract administration (150 and 250 mg/kg body wt/day) normalization in these parameters were obtained with signifies the reduced stress level (Table VI), remodeling of tissue architecture, and hence reduced concentration of ALT and AST. The similar results were obtained in the previous studies [32].

Hepatic tissue plays a major role in homeostasis of glucose during starvation and post prandial. After post prandial, hepatocytes stores glucose in the form of glycogen under the influence of insulin with the help of glucokinase (GK) activity[33], while in starvation period, hepatocytes release glucose through glycogenolysis and gluconeogenesis with the help of Glucose-6 phophatase activity [34]. In the present investigation, Glucokinase enzyme level decreased along with the decrease in Glycogen content but Glucose-6-phosphatase enzyme activity showed a elevated level in STZ induced diabetic mice [35] (Table VII). Following the administration of *Annona* extract of different doses (250 and 150 mg/kg body wt/day), there was a marked increase of glycogen content, and Glucokinase activity ($p<0.01$) whereas, significant decrease in the Glucose-6-phosphatase concentration in diabetic rats fed with the herbal extract as compared to diabetic control, marks the ameliorating properties of the plant. Results in case of the herbal extract were more significant and effective as compared to synthetic drug, Rosiglitazone in the experiment. As glucokinase and G-6-phosphatase activities is regulated by insulin along with glycogen formation so it may be argued that *A. squamosa* extract stimulates β-cell of pancreas to synthesis and secrete insulin, which decrease the G-6-phosphatase activity and increase GK activity in Liver, also promoting glycogen formation, the findings corroborates with the previous finding [36].

V. Conclusion

Diabetes is a chronic disorder in metabolism of carbohydrates, proteins, and fat due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance. The efficacy of medicinal plants in diabetic management is of great interest due to their beneficial effects on metabolic profile. In the present study, the herbal extract of *Annona squamosa* Linn. (*Annonaceae*) has been evaluated against diabetes. *Annona squamosa* leaf extract exhibited hypoglycemic activity in diabetic mice but not in normal

mice. It reduced both the blood glucose as well as lipid levels. It also has a positive improvement in the liver and kidney functions as compared to Rosiglitazone which are normally affected in the diabetic untreated mice. It could be concluded that the herbal extract of *Annona squamosa* could be better alternative against the synthetic drugs for reducing the diabetic complications in a natural way. However, translational research is required on human with large sample size because the route of administration and physiology of human differs with that of the mice.

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Conflict of interest

Authors declare no conflict of interest regarding publication or any other activity related to this article.

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