WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 7, 759-769. Research Article ISSN 2277– 7105

A STUDY OF ETHANOLIC EXTRACT OF ANNONA SQUAMOSA LEAVES ON BLOOD GLUCOSE LEVELS AND BODY WEIGHT CHANGES IN DIABETIC RATS

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Article Received on
13 April 2022,

Revised on 03 May 2022,
Accepted on 23 May 2022

DOI: 10.20959/wjpr20227-24342

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ABSTRACT

Diabetes is one of the most common chronic diseases. Now a days, so many people have been suffering with Diabetes mellitus. It is a metabolic disorder in which glucose levels are increased in the blood. This hyperglycemic condition effects the metabolic activities in the body and cause symptoms like extreme thirst, frequent urination, blurred vision, Fatigue, Weight loss, Poor wound healing, Dry or itchy skin, Impotence, Recurrent Infections *etc.* In Diabetes, the oxidative stress enhances the free radical formation which leads to chronic disorders in which glucose levels will be enhanced and body weight changes will occur. In our study, we have used the ethanolic extract of *Annona squamosa* leaves on diabetic rat, these leaves decreased the blood glucose levels nearer to the referral drug.

KEYWORDS: Diabetes mellitus, Free radicals, Oxidative stress, *Annona squamosa*, Streptozotocin. Glibenclaimade.

INTRODUCTION

Diabetes mellitus

Diabetes is a condition in which glucose levels are increased in the blood. This hyperglycemic condition effects the metabolic activities in the body. Liver is a major producer of glucose, it releases glucose from the breakdown of glycogen and also from intermediates of carbohydrate, protein and fat metabolism. The amount of glucose in the blood stream is regulated by insulin. The glucose levels normally ranging from 70 to 110

mg/dl. The rise in blood glucose levels are due to diet, breakdown of glycogen and hepatic synthesis of glucose. Glucose absorption across the hydrophobic gut wall requires specialized glucose transporters (GLUTS) i.e., GLUT2 and GLUT5.

Annona squamosa

Figure 1: Annonus squamosa plant.

Annona squamosa, also called **sugar-apple** or **sweetsop** is a species of *Annona.* The roots, bark, fruits i.e., both Ripened and Dried unripen fruit, seeds have several medicinal properties. As per ancient literature, more than 800 plants are reported to have antidiabetic properties.[1] Ethnopharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycemic activity.[2] Several studies revealed that the use of young leaves of *A. squamosa* along with the seeds of *Piper nigrum* for the management of diabetes mellitus.[3,4]

The previous study have demonstrated the antidiabetic effect of *A. squamosa* in streptozotocin (STZ)-induced diabetes mellitus in rats.[5] Therefore, the present study aimed to examine the oral administration of *A. squamosa* ethanolic extract of leaves on the STZ induced diabetic rats.

Oxidative stress

Oxidative stress has been shown to play a role in the causation of diabetes mellitus. Antioxidants have been shown to have a role in the alleviation of diabetes mellitus.[6] In diabetes mellitus, Oxygen Free Radicals (OFRs) are generated by stimulating H_2O_2 *in-vitro,* as well as *in-vivo,* in pancreatic *ß*-cells.[7] OFR-scavenging enzymes can respond to conditions of oxidative stress with a compensatory mechanism that increases the enzyme activity in diabetic rats.[8]

Free radical formation

Free radical is any atom (e.g., oxygen, nitrogen) with at least one unpaired electron in the outermost shell, and is capable of independent existence. Free radicals are highly reactive due to the presence of unpaired electron(s). Any free radical involving oxygen can be referred to as Reactive Oxygen Species (ROS). Singlet oxygen can transfer the energy to a new molecule and act as a catalyst for free radical formation. The molecule can also interact with other molecules leading to the formation of a new free radical.

Oxygen-derived free radicals have been implicated in the pathophysiology of diabetes mellitus (Giugliano *et al*., 1996). superoxide anion is the primary radical formed by the reduction of molecular oxygen which may lead to secondary radicals or reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radical (Grisham & McCord, 1986;). It was found that increased oxidative stress has been involved in the pathogenesis and progression of diabetic tissue damage (Jang *et al.*, 2000 & Katusic, 1996).

MATERIALS AND METHODS

Preparation of extracts

The leaf powder of *Annona squamosa* was extracted according to Evans and Trease, 1996. The leaf powder of *Annona squamosa* was extracted with 5 volumes of 95% Ethyl alcohol. The extract obtained was distilled and concentrated under reduced pressure at temp (40^0C) in the rota evaporator. A dark green, semi solid residue was obtained, stored at 4^0C and used for further studies.

Selection, Care and Maintenance of experimental animals

For our study we have used, Pathogen free, Wister strain male albino rats of young age 3 months and body weight 160 $\pm$ 20g. The rats were housed in clean polypropylene cages under hygienic condition with photo period of 12 hours light and 12 hours dark. The rats were fed with standard laboratory chow (Hindustan Lever Ltd., Mumbai) and water *ad libitum.*

Induction of diabetes

Diabetes was induced in these rats by single intra peritoneal injection of freshly prepared streptozotocin (50 mg/kg b.w.) dissolved in ice cold 0.1 M citrate buffer (pH 4.5) after allowing the rats for overnight fasting for 12-15 hours as per the method of Rakieten *et al.*, (1963). Since STZ is capable of producing fatal hypoglycemia as a result of massive

pancreatic insulin release due to destruction of βcells, 8 hrs after STZ administration the rats were kept for next 24 hours on 15% glucose solution to prevent hypoglycemia. Diabetes was assessed by determining the fasting blood glucose after 48 hrs of injection of STZ. The blood glucose levels in STZ rats were increased markedly higher levels than normal. After a week, when the condition of diabetes was stabilized, rats with marked hyperglycemia (blood glucose level ≥ 250g) were selected and used for the study.

The protocol of this study was submitted to Institutional Animal Ethics committee and approved in its resolution: (Resolution Number438/01/a/CPCSCA/IAEC/SVU/KSR-1 dt: 11-9-2008)

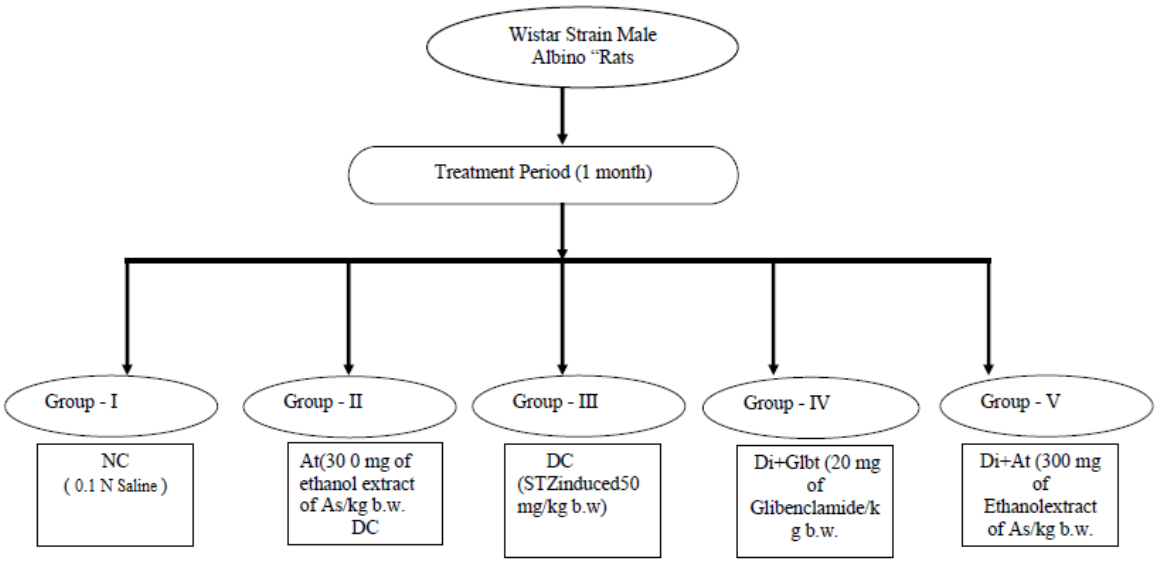


Figure 2: Experimental Design Chart: Group of animals used in our study.

Estimation of blood glucose

Blood glucose levels were estimated frequently by using Accuchek glucometer.

Checking of body weight changes

Body weights of the following were recorded before and after treatments at an interval of one week till the completion of the experimental period.

- Control (0.1 N Saline)
- *Annona* treated
- Diabetic
- Glibenclamide treated
- Diabetic rats with *Annona* supplementation

RESULTS AND DISCUSSION

In the present study, the blood glucose levels were measured in units of mg/dL, in all the experimental groups i.e., in control, Annona treated, diabetic, Glibenclaimade treated diabetic rats and Annona treated diabetic rats. The levels were increased in diabetic rats and decreased in Annona treated diabetic rat which are mentioned in Table:1, 2, 3 and Fig: 1, 2 and 3.

Table 1: Changes in Blood glucose levels (mg/dl) in Normal control (NC) Annona treated (At) (300mg/body weight), diabetic control (DC) (STZ-50mg body weight), diabetic+Glibenclamide treated(20mg/kg) (Di+Glbt) Diabetic + Annona treated rats (Di+ At).

Blood Glucose	Treatment									
	NC		At		DC		Di+Glbt		Di+At	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Mean Sd	85 ±0.81	87 ±1.29	84 ±1.63	79 ±3.55	84* ±27.46	300* ±10.59	84** ±6.817	196** ±2.88	84** ±3.03	204** ±7.31
Percent change										

All the values are mean ± SD of six individual observations

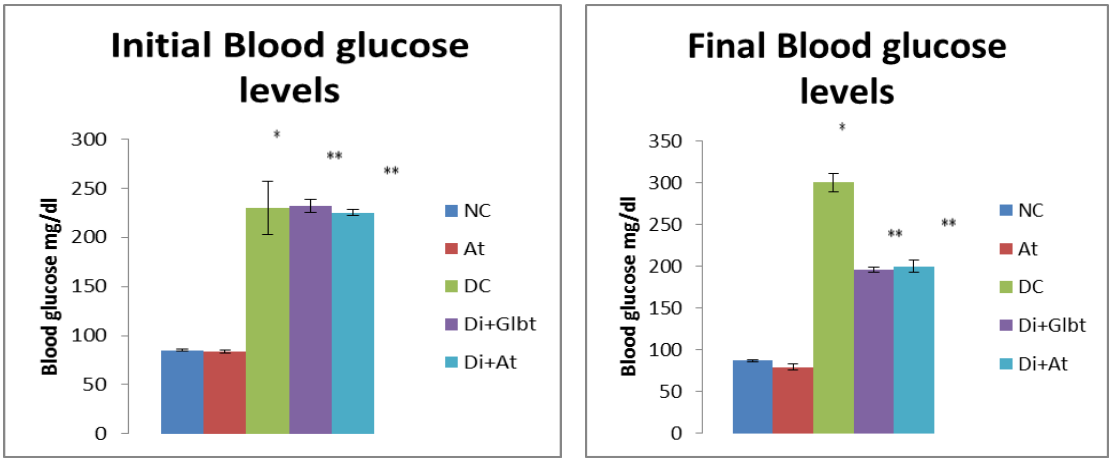
Values in the parentheses denote per cent change over normal control Values are significant compared to normal control at *(P<0.05) ** (P<0.01)

Table 2: One way ANOVA for Blood Glucose (Initial).

Between treatments			Within Treatments			(a)+(b)	(x)+(y)	F	P
Df(a)	Sum of Squares (x)	Mean Squares	Df (d)	Sum of Squares (y)	Mean squares				
4	7.133	1.783	25	51.167	2.047	29	58.300	0.871	0.495

Table 3: One way ANOVA for Blood glucose (Final).

Between treatments			Within Treatments			(a)+(b)	(x)+(y)	F	P
Df(a)	Sum of Squares (x)	Mean Squares	Df (d)	Sum of Squares (y)	Mean squares				
4	20311 1.533	5077 7.883	25	567.833	22.713	29	20367 9.367	2.236	.000



a) Intial Blood Glucose levels **b) Final Blood Glucose levels**

Figure 3: Effect of NC, At, DC (STZ), Di+Glbt and Di+At on a) Intial Blood Glucose levels b) Final Blood Glucose levels content in kidney tissue of male albino rats. The Values marked with *(P<0.05) ** (P<0.01).

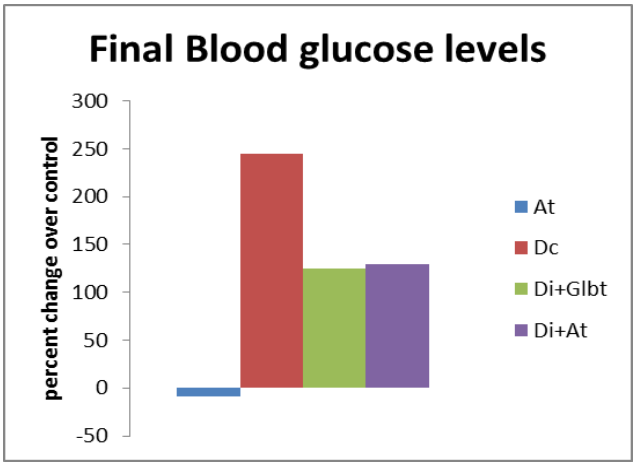


Figure 4: Percentage graph for Blood Glucose levels – Percentage change in Kidney. Final Blood Glucose levels content in Experimental rats over the Control.

The significant increase in blood glucose levels in diabetic rats could be due to destruction of pancreatic β cells by STZ. STZ induces diabetes through the generation of oxygen free radicals. (Wogaieb and Godin, 1987). The elevation of glucose in STZ treated rats was due to an oxidative stress in pancreatic islets DNA (Omamto *et al.*, 1981).

In experimental diabetes, enzymes of glucose and fatty acid metabolism are markedly altered; hence blood glucose levels were increased (Gottried and Rosenberg, 1973; Sochor *et al.*, 1985). An increased hyperglycemia has been reported to induce oxidative stress due to glycation of proteins and accumulation of polyols (Low *et al.*, 1997).

Administration of *A. squamosa* leaves extract to STZ-induced diabetic rats resulted in a significant reduction in blood glucose level. The possible mechanism of hypoglycaemic action of ethanolic extract of *A. squamosa* leaves, may be through potentiation of pancreatic secretion of insulin from β-cell of islets.

In the present study, the body weights were observed at an interval of one week for the period of one month in control, *Annona* treated, diabetic, diabetic rats with Glibenclaimade treatment and diabetic rats with *Annona* treatment. The body weights of individual rats were normalized to 200grams for young rats. In the present study we observed that the body weights were increased in control rats, diabetic rats with Glibenclaimade treatment and diabetic rats with *Annona* treatment. Where as in *Annona* treated and diabetic rats of the body weights were decreased. In calculated growth changes showed a substantial growth in rats and a similar feature was observed with *Annona* treatment in diabetic rats. (Table: 4,5 and 6, Fig:4,5 and 6).

Table 4: Changes in Body weights levels (gm/kg) in Normal control (NC) Annona treated (At) (300mg/body weight), Diabetic control (DC)(STZ-50mg body weight), Diabetic+Glibenclamide treated(20mg/kg) (Di+Glbt) Diabetic + Annona treated rats (Di+ At).

Body weight	Treatment									
	NC		At		DC		Di+Glbt		Di+At	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Mean Sd	209 ±5.19	229 ±5.20	226** ±3.46	199** ±3.63	216 ±4.86	164** ±1.29	226** ±3.26	205* ±4.30	215 ±3.26	192** ±3.43
Percent										
change										

All the values are mean ± SD of six individual observations

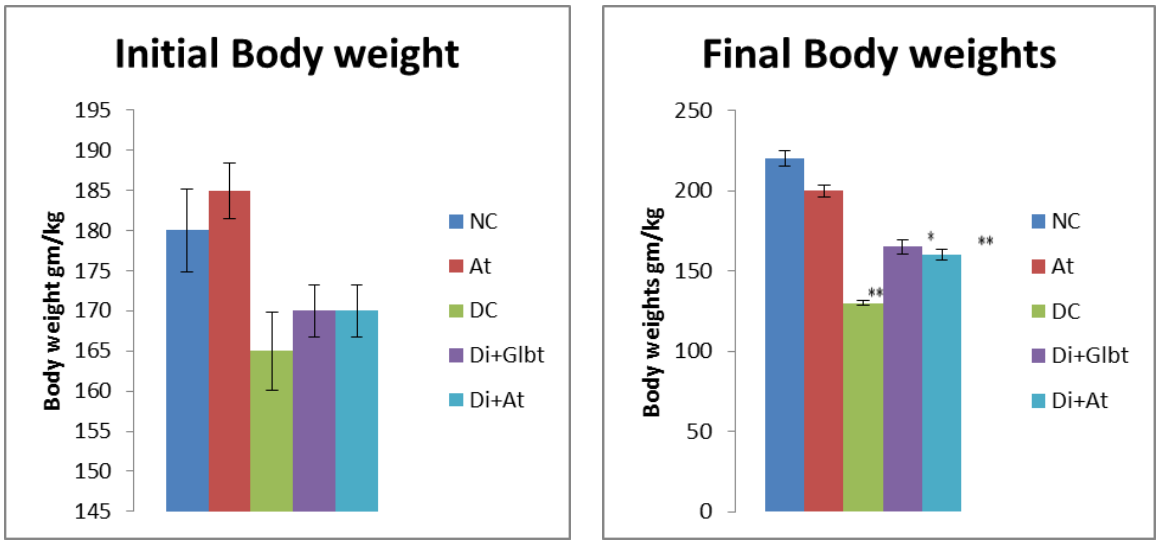
Values in the parentheses denote per cent change over normal control Values are significant compared to normal control at *(P<0.05) ** (P<0.01)

Table 5: One way ANOVA for Body weight (Initial).

Between treatments			Within Treatments			(a)+(b)	(x)+(y)	F	P
Df(a)	Sum of Squares (x)	Mean Squares	Df (d)	Sum of Squares (y)	Mean squares				
4	1389.800	347.450	25	963.167	38.527	29	2352.967	9.018	.000

Table 6: One way ANOVA for Body weight (Final).

Between treatments			Within Treatments			(a)+(b)	(x)+(y)	F	P
Df(a)	Sum of Squares (x)	Mean Squares	Df (d)	Sum of Squares (y)	Mean squares				
4	12866.467	3216.617	25	702.333	28.093	29	13568.800	114.498	.000



a) Intial body weight **b) Final body weight**

Figure 5: Effect of NC, At, DC (STZ), Di+Glbt and Di+At on a) Intial body Weight b) Final body weight content in kidney tissue of male albino rats.

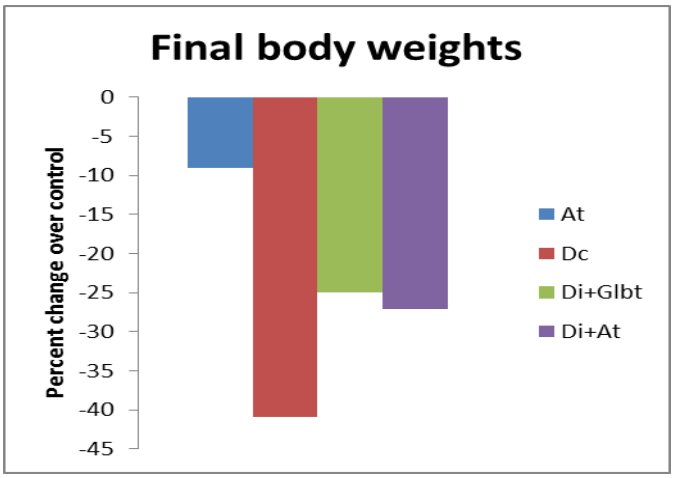


Figure 6: Percentage graph for Final Body Weights–Percentage change in Kidney, Final Body Weight content in Experimental rats over the Control.

In the present study, the diabetic rats showed decreased level of body weights. The decrease in the body weight in diabetic rats clearly shows a loss or degradation of structural proteins. Due to diabetes the structural proteins are known to contribute for the

body weight (Rajkumar & Govindarajulu, 1991). STZ induced diabetes is characterized by a severe loss in body weight. (Chen & lanuzzo, 1982). The control diabetic animals showed a significant decrease in body weight compared with normal rats (AI-Amin 2006). Changes in body weight in adult and non-adult diabetic rats varied. Since the non-adult diabetic rats are in the growing age, diabetic loss of weight is not seen in them and they even show a slight weight gain. Weight loss during diabetes is mainly related to urinary glucose excretion because cells become to use glucose. Another factor could be also the osmotic diuresis resulting in hyper osmotic dehydration (Kaplan *et al.*, 1982).

CONCLUSIONS

In our study, concentrations of oxidative stress was increased in diabetic rats, indicating an increase in the generation of free radicals. The present finding indicates significantly increased lipid peroxidation of rats exposed to STZ and its attenuation by *A. squamosa* treatment. This suggests that the protective role of *A. squamosa* leaf extracts could be due to the antioxidative effect of flavonoids present in the leaf, which in turn act as strong superoxide radicals and singlet oxygen quenchers. A number of investigations have also reported that alkaloids, flavonoids like rutin and hyperoside of Annona leaves have hypoglycemic and other pharmacological actions in various experimental animal models (Gupta *et al., 2005*; Yoganarasimhan & Seetharaman, 2000).

Kaleem *et al.,* (2006) reported that Annona possess hypoglycemic effect. One of the consequences of hyperglycemia is increased metabolism of glucose by sorbitol pathway. Besides this, other path ways such as fatty acid and cholesterol biosynthesis favor hyperglycemia (Vijaykumar *et al.,* 2006). Hyperglycemia is currently considered to be primarily responsible for the auto- oxidative glycosylation, formation of hydro peroxides and free radicals, in particular the hydroxyl radical and low-density lipoprotein oxidation (Hunt *et al.*, 1990).

In our study, Blood glucose levels were increased in diabetic, but in *Annona* treated alone and *Annona* treated diabetic group the blood glucose levels were decreased. This may be due to the antidiabetic compounds present in ethanolic extract of *Annona* leaves. We have also observed body weight changes in all experimental groups. In *Annona* treated, diabetic group the body weights were decreased. This may be due to mobilization of body reserves like adipose tissue or fat

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