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Research Article

ANTI-HYPERGLYCEMIC EFFECT *ANNONA RETICULATA* L. LEAVES ON EXPERIMENTAL DIABETIC RAT MODEL

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ABSTRACT

Context: *Annona reticulata* Linn (Annonaceae) commonly known as bullock's heart is widely distributed all over India and leaves are used as insecticides, anthelmintic, styptic and are also used externally as suppurant.
Objective: The present study has been designed to determine the effectiveness of Hydro-Alcoholic extract of leaves of *Annona reticulata* L. (HAAR) for potential hypoglycemic activity against streptozotocin (STZ) induced hyperglycemic rats.
Materials and methods: Hyperglycemia was induced in rats by administration of multiple low doses (40 mg/kg) of Streptozotocin (STZ) to the overnight fasted rats for five consecutive days intraperitoneally. After 12days of STZ administration, the fasting blood glucose levels (FBG) were measured and the rats with FBG level >250 mg/dL were considered to be diabetic and were used in the study. The study was undertaken by using the plant extract at dose levels of 200 mg/kg and 400 mg/kg (as test), p.o. and standard drug as Metformin (300 mg/kg) in both normoglycemic and streptozotocin induced hyperglycemic animals. Hypoglycemic potential was assessed by the measurement of fasting blood glucose, Lipid Profile (total cholesterol, triglycerides), physical parameters (body weight, food and water intake) and Oral Glucose tolerance test.
Results and discussion: Results of the studies showed that, the fasting blood glucose level in hyperglycemic and in oral glucose tolerance test, showed a significant ($p<0.05$) decrease at defined time points, while the observed biochemical and physical parameters showed a good agreement with hypoglycemic property of the extract.
Conclusion: The present study suggests that *Annona reticulata* leaves possess potent glucose lowering effect.

Key Words: *Annona reticulata,* Streptozotocin, Metformin, Hyperglycemia, Diabetes

INTRODUCTION

Annona reticulata Linn, commonly known as bullock's heart or raamphal plant, is widely distributed all over India and are tall, with many branches, bearing nutritious fruits. The leaves are used as insecticides, anthelmintic, styptic and are also used externally as suppurant. The bark as a powerful astringent is used as antidysentric and vermifuge. Root bark, leaves and stem possess isoquinoline alkaloids[1]. The present study has been designed to determine the effectiveness of Hydro-Alcoholic extract of leaves of *Annona reticulata* L. (HAAR) for potential hypoglycemic activity, if any, against normoglycemic and streptozotocin induced hyperglycemic rats.

Material and Methods

Collection of plant material & preparation of extract

The aerial parts of *A. reticulata* L. were collected in the month of July – August 2010, from the rural area of the dist. Cuttack, Odisha, India and authenticated by Dr. Kshetra Mohan Das, Senior Scientist of the Central Rice Research Institute, Cuttack, Odisha, India and a specimen voucher (Specimen No. 10-11/SPS/SOAU) has been kept in the University for future reference. The leaves were shade dried and made into coarse powder. The powdered material was initially defatted with petroleum ether followed by 72-hours extraction with 1: 1 mixture of methanol and water using cold maceration process for 72-hours. The extract was filtered and concentrated by rotary evaporator and kept in vacuum desiccators until use. The yield of the extract was 20.5 % w/w with respect to dried powder.

Preparation of Interventions

The Hydro-Alcoholic Extract of *Annona reticulata* (HAAR) (at dose levels of 200 mg/kg and 400 mg/kg) was suspended in distilled water using 25% Tween 20 as suspending agent. The standard drug Metformin (300mg/kg) was also prepared in a similar manner. Suspension of distilled water and 25% Tween 20 was used as solvent treatment throughout the study at a dose of 2 ml/kg. The solvent, test samples and standard drugs were administered by oral route based on dose and corresponding weight of the animals.

Experimental animals

Healthy Wistar albino rats (150 – 250 gm body weight) supplied by Central Animal House of School Of Pharmaceutical Sciences, SOA University, Bhubaneswar, India were used in the experiments. The animals were acclimatized to laboratory conditions for one week before commencement of experiment. The study was approved by University Animals Ethics Committee (Regd. No. 1171/C/08/CPCSEA).

Induction of diabetes

Diabetes was induced by administration of multiple low doses (40 mg/kg) of Streptozotocin (STZ) to the overnight fasted rats for five consecutive days intraperitoneally. STZ solution was prepared freshly in ice- cold citrate buffer (0.01 M, pH 4.5)[2]. After 12days of STZ administration, the fasting blood glucose levels (FBG) were measured and the rats with FBG level >250 mg/dL were considered to be diabetic and were used in the study.

Blood glucose level & biochemical parameters measurement

Glucose level was estimated by using Glucometer (One Touch Horizon, Lifescan, Johnson and Johnson Company). On terminating the dosing, the rats were fasted for 12 h, sacrificed by decapitation, blood samples were collected by standard method for estimation of serum triglycerides and cholesterol by using commercially available diagnostic kit.

Acute toxicity study

Healthy Wistar albino rats of either sex starved overnight, were divided into five groups (n=4). Group I-IV animals were orally fed with HAAR in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 g/kg. b.w., while group V (untreated) served as control. The animals were observed continuously for the first 2 h for any gross change in behavioral, neurologic and autonomic profiles or any other symptoms of toxicity and mortality if any, and intermittently for the next 6 h and then again at 24 h , 48 h and 72 h for any lethality or death. One-tenth and one-fifth of the maximum safe dose of the extract tested for acute toxicity were selected for the experiment[3].

Oral glucose tolerance test (OGTT)

Oral glucose tolerance test has been conducted as per established methods[4, 5]. The apportionment of animals into different groups and administration of different interventions has been done as explained in single oral dose hypoglycemic study. The normal rat groups were loaded with glucose (2 g/kg/p.o.) 30 minutes after administration of interventions; whereas diabetic rats were loaded with glucose (5 g/kg) 1hr after administration of interventions. Blood samples were collected from the tail vein at 0, 30, 60, 120 and 180 minutes after glucose loading and glucose levels were measured immediately.

Single dose hypoglycemic study

The effect of single dose administration of HAAR on blood glucose level was studied by following previously published methods [4,6,7]. The normal and diabetic rats were divided into different groups and treatments were given as follows; Group I (Normal rats treated with solvent 2 ml/kg), Group II (Normal rats treated with HAAR 200 mg/kg), Group III (Normal rats treated with HAAR 400 mg/kg), Group IV (Normal rats treated with Metformin 300 mg/kg), Group V (Diabetic control rats treated with solvent 2 ml/kg), Group VI (Diabetic rats treated with HAAR 200 mg/kg), Group VII (Diabetic rats treated with HAAR 400 mg/kg), Group VIII (Diabetic rats treated with Metformin 300 mg/kg). After 12hrs of fasting, each group of animals received single dose of either solvent or HAAR (200 mg/kg and 400 mg/ kg) or Metformin 300 mg/kg and the experiment was carried out under fasting condition. Blood sample was collected from tail vein at 0, 1, 2, 4, 6 and 12 hrs after administration of intervention to estimate blood sugar.

Multiple dose hypoglycemic study

Multiple dose study has been conducted by following methods established previously [4, 6]. The apportionment of animals into different groups similarly as it has been done in previous experiments. Each group either received solvent (2ml), or HAAR 200 mg/kg, or HAAR 400 mg/ kg, or Metformin 300 mg/kg everyday 30 minutes before food throughout the experiment duration.

In case of normal rats the treatment was given continuously for 9 days and fasting blood sample was collected on day 0, 1, 3, 6 and 9 for estimating blood sugar. On 10th day blood sample was collected for estimation of serum lipid profile. During the 10 days of observation of the rats were observed for any changes in the body weight relative to day 0, i.e. before the start of the treatment. In case of diabetic rats the treatment was given continuously for 14 days and fasting blood sample was collected on day 0, 1, 3, 6, 9 and 14 for estimating blood sugar. Blood sample was collected for estimation of serum lipid profile on day 15. In case of diabetic rats; food and water intake habits of the rats has been observed.

Statistical analysis

Results are expressed as Mean ± SE. The data are analyzed by one way ANOVA followed by Turkey – Kramer Multiple Comparison test. Confidence Interval has been considered as 95% and $p < 0.05$ are considered significant.

Results

Effect of HAAR in OGTT

The result of Oral Glucose Challenge test in Normal and Hyperglycemic rats has been presented in Table 1 & Table 2 respectively. In normal rats the maximum percentage increase in blood glucose level in comparison to base line are 84.99 %, 52.51 %, 40.43% & 19.56 % with respect to treatment with Solvent, HAAR 200mg/kg treated group, HAAR 400 mg/kg and Metformin. The percentage decrease in glucose level from the maximum at end of the experiment is 6.43%, 34.55%, 36.79% & 29.82% in case of Control Group, HAAR 200mg/kg treated group, HAAR 400 mg/kg treated group and Metformin treated group respectively. Similarly in case of hyperglycemic rats; maximum percentage increase in blood glucose level in comparison to base line in Control Group, HAAR 200mg/kg treated group, HAAR 400 mg/kg treated group and Metformin treated group is 35.34 % , 31.79 %, 31.39 % & 20.81 % respectively. The percentage decrease in glucose level at the end of the experiment is 10.89 %, 16.08%, 30.47 % & 36.33 % in case of Control, HAAR 200mg/kg, HAAR 400 mg/kg and Metformin treated group respectively from the maximum level attended.

Effect of HAAR on blood glucose level of Normal rats

Table 3 and Table 4 show the effect of HAAR on blood glucose level of Normoglycemic rats. In case of single dose study (Table 3), HAAR at both the dose levels did not show any significant ($p < 0.05$) decrease in blood sugar level even at 12th hr of the observation where as the standard drug metformin showed the effect significantly ($p < 0.01$) from 2nd hr of the observation and onwards. The percentage decrease in blood glucose level at the end of the study (12th hr) with respect to 0 hr is recorded as 1.94%, 6.43% & 25.31% with administration of HAAR 200 and 400 mg/kg & Metformin 300 mg/kg respectively. Similarly in case of multi dose administration study (Table 4); even on 9th day of observation HAAR at both the dose levels do not decrease blood glucose level significantly ($p < 0.05$) when compared with the control group, where as the standard drug metformin (300 mg/kg) decreases blood glucose level significantly ($p < 0.01$) from 3rd day of observation till the end of the study (Day 9). The percentage decrease in blood glucose level caused by HAAR 200 mg/kg and 400 mg/kg and metformin 300 mg/kg is 8.81%, 10.99% & 33.68% respectively at the end of study.

Table 1: - Oral Glucose Challenge in Normal Rat

Treatment↓	Fasting Blood Glucose (mg/dL)						% change from Highest value Observed
	-30 Mnts	Base Line	30 Mnts	60 Mnts	120 Mnts	180 Mnts	
Normal Control	94.83±1.58	96.67±2.12	178.83±1.74	175.17±2.43	171.83±3.32	167.33 ± 3.28	- 6.43
HAAR 200mg/kg	98.17±0.60	96.50±0.56	129.83±4.23***	147.17±4.52***	106.83 ± 3.21***	96.33 ± 3.03***	- 34.55
HAAR 400mg/kg	98.5±0.43	94.83±1.01	116.17±3.27***	133.17±4.07***	92.83 ± 1.14***	84.17 ± 1.52***	- 36.79
Metformin 300mg/kg	102.33±1.73	96.33±2.19	115.17±2.10***	95.50±4.47***	83.50±3.48***	80.83±2.73***	- 29.82

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test; *p<0.05, **p<0.01, ***p<0.001 *vs*. Control Group; "-" sign indicates decrease in the value from the initial value

Table 2: - Oral Glucose Challenge in Hyperglycemic Rats.

Treatment↓	Fasting Blood Glucose (mg/dL)						% change from Highest Value Observed
	-1Hrs	Base Line	30 Mnts	60 Mnts	120 Mnts	180 Mnts	
Diabetic Control	308.67±16.78	306.5±16.80	414.83±21.09	409.17±20.84	398.17±18.95	369.67±19.58	- 10.89
HAAR 200mg/kg	305.5±1.23	276.83±1.11	318.17±10.65***	364.83±4.04	341.67±3.14*	306.17±4.02**	- 16.08
HAAR 400mg/kg	306.67±0.76	260.17±4.59	293.67±12.66***	341.83±12.88**	280.5±12.53***	237.67±7.49***	- 30.47
Metformin	304.5±7.85	234.67±7.69	283.5±9.91***	238.00±10.79***	200.33±8.62***	180.5±6.57***	- 36.33

300mg/kg							

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test; *p<0.05, **p<0.01, ***p<0.001 *vs*. Control Group; "-" sign indicates decrease in the value from the initial value

Table 3 : - Effect Of Single Dose Of Extract(S) On Blood Glucose Of Normoglycaemic Rats

Treatment ↓	Fasting Blood Glucose (mg/dL)						% Change From 0Hr
	0hr	1 hr	2 hrs	4 hrs	6 hrs	12 hrs	
Normal Control	96.50±2.09	96.66±1.56	96.50±1.57	97.83±1.42	96.60±2.09	98.33±1.52	1.89
HAAR 200mg/kg	94.33±0.99	93.33±0.88	92.00±0.86	90.67±0.67	89.33±0.76	92.50±0.43	- 1.94
HAAR 400mg/kg	98.5±1.432	96.83±1.60	95.50±1.52	93.67±1.23	91.50±1.23	92.17±0.54	- 6.43
Metformin 300mg/kg	96.16±3.57	90.5±3.16	78.50±2.49***	75.66±3.59***	72.66±3.30***	71.83±2.73***	- 25.30

Values are expressed as Mean ± SEM; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test; *p<0.05, **p<0.01, ***p<0.001 *vs*. Control Group; "-" sign indicates decrease in the value from the initial value.

Table 4 : Effect Of Multi Dose Of Extract(S) On Blood Glucose Of Normoglycaemic Rats

Treatment ↓	Fasting Blood Glucose (mg/dL)					% Change From Day - 0
	Day 0	Day 1	Day 3	Day 6	Day 9	
Normal Control	94.50±1.544	97.83±1.87	97.00±0.73	95.83±1.58	95.83±1.96	1.41
HAAR 200mg/kg	98.33±2.45	97.17±2.04	94.67±2.12	91.67±2.12	89.67±2.17	- 8.81
HAAR 400mg/kg	98.67±2.01	97.17±1.91	93.67±1.87	90.33±2.22	87.83±2.12	- 10.99
Metformin 300mg/kg	96.5±3.75	90.16±2.47	81.00±2.39***	73.83±1.97***	64.00±1.97***	- 33.68

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test; *p<0.05, **p<0.01, ***p<0.001 *vs*. Control Group; "-" sign indicates decrease in the value from the initial value

Effect of HAAR on blood glucose level of Hyperglycemic rats

From the results of single dose administration study (Table 5); it's evident that in all treated groups the reduction in blood glucose level is significant ($p < 0.001$) from 1st hr of the observation till the end of the experiment when compared with control group. At the end of the study the percentage decrease in blood glucose is 40.05%, 47.36% and 43.06% by treatment with HAAR 200 mg/kg and HAAR 400 mg/kg and metformin 300 mg/kg respectively. In case of multiple administration of the test extract and metformin, it's has been observed that there is significant ($p < 0.001$) reduction in blood glucose level in the 1st day of observation when compared with control group. The percentage reduction in blood glucose level at end of the study caused by HAAR 200 mg/kg and HAAR 400mg/kg is 47.80% and 54.73% respectively. The percentage reduction caused by standard drug metformin is 55.25%. All the studies carried out on hyperglycemic rats show that the effect of HAAR is similar to that of standard drug metformin.

Effect on Basic Lipid Profile

Effect of HAAR on cholesterol and Triglyceride parameters of normal and hyperglycemic rats has been observed and cited in Table 7. Administration of HAAR at both the dose levels for 9days to normal rats, it has been observed that the test extract decreases the triglyceride as well as cholesterol level significantly (p<0.05) only at 400mg/kg dose when compared with normal control group. In case of hyperglycemic rats the observed parameters have been remarkably corrected significantly (p<0.001) when compared with solvent treated group.

Effect on body weight

The results effect of HAAR on body weight of normal rats and hyperglycemic rats are depicted in Table 8 & Table 9 respectively. In case of normal rats it has observed that there is no significant difference of change in body weight between the extract treated groups and the control group. The percentage increase in body weight in Control group, HAAR 200 mg/kg and HAAR 400 mg/kg treated group is found as 6.23%, 5.58% and 6.76% respectively. So the extract doesn't affect the change in body weight of normal animals. Treatment with metformin showed decrease in the body weight of normal rat significantly ($p < 0.01$) when compared with control group. The percentage decrease in body weight is computed as 15.4%. It's well known that metformin may cause decrease in body weight in non-diabetics by reducing hunger [8].

The extract showed protection against body weight loss in hyperglycemic rats (Table 9), which is a characteristic feature of anti-hyperglycemic activity of the extract, while the standard drug metformin showed no significant change of the body weight with respect to control group of animals. The decrease in body weight in hyperglycemic rats treated with metformin may be attributed to decreased calorie intake [9].

Effect on water intake and food intake of hyperglycemic rats

The effects the extract on water and food intake habit of hyperglycemic rats are depicted in Table 10 & Table 11 respectively. HAAR at tested dose levels, considerably reduced food and water intake of hyperglycemic rats in comparison to control group. At the end of 2nd week, the extract treated groups showed significant ($p < 0.001$) decrease in water and food intake in comparison to untreated group, with an extent of 38.13% (HAAR 200 mg/kg); 49.04% (HAAR 400 mg/kg) in case of water intake and 46.53 (HAAR 200 mg/kg); 52.59% (HAAR 400 mg/kg) in case of food intake relative to Week-0 (Before Administration of Test products).

Table 5: - Effect Of Single Dose Of Extract(S) On Blood Glucose Of Hyperglycaemic Rats

Treatment ↓	Fasting Blood Glucose (mg/dL)						% Change From 0Hr
	0hr	1hr	2hr	4hr	6hr	12hr	
Diabetic Control	361.83±2.60	359.66±5.93	350.83±3.40	345.33±2.60	333.33 ± 2.72	328.66 ± 2.69	- 9.17
HAAR 200mg/kg	372.83±2.44	325.33±7.81***	279.5±4.87***	260.17±3.93***	247.5±2.08***	223.5±2.09***	- 40.05
HAAR 400mg/kg	372.67±3.16	306.33±5.06***	257.33±5.45***	240.5±4.62***	224.33±5.78***	196.17±2.93***	- 47.36
Metformin 300mg/kg	369.66±4.74	290.83±4.82***	259.83±3.04 ***	236.83±2.97***	214.16±4.11***	210.50±3.74***	- 43.05

Values are expressed as Mean ± SEM. (n = 6) One Way ANOVA followed by Turkey – Kramer Multiple Comparison test *p<0.05, **p<0.01, ***p<0.001 *vs*. Control Group; "-" sign indicates decrease in the value from the initial value

Table 6: Effect Of Multi Dose Of Extract(S) on Blood Glucose Of Hyperglycaemic Rats.

Treatment↓	Fasting Blood Glucose (mg/dL)						% Change From
	Day 0	Day 1	Day 3	Day 6	Day 9	Day 14	Day - 0
Diabetic Control	361.33±2.11	372.16±2.81	389.83±2.94	401.16±3.54	398.50±2.57	401.66±3.30	11.16
HAAR 200mg/kg	364.33±3.82	340.33±2.81***	310.83±3.77***	281.83±3.95***	235.67±1.89***	190.17±1.83***	- 47.80
HAAR 400mg/kg	366.33±5.02	330.67±4.26***	291.67±3.48***	255.67±2.73***	219.33±2.17***	165.83±2.70***	- 54.73
Metformin 300mg/kg	370.16±5.79	264.83±4.41***	246.66±4.37***	240.00±4.68***	202.66±2.26***	165.66±1.86***	- 55.25

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test; *p<0.05, **p<0.01, ***p<0.001 *vs*. Control Group; "-" sign indicates decrease in the value from the initial value

Table 7: Effect of Multi Dose of Extract(S) On Basic Lipid Profile

Animal →	NORMOGLYCAEMIC RATS		HYPERGLYCAEMIC RATS	
Treatment ↓	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)
Normal Rats	80.89 ±2.47	61.49 ±1.59	142.95 ± 6.62	93.70 ± 4.33
HAAR 200mg/kg	76.39 ±2.42	55.52 ±2.36	80.30 ± 1.65***	39.99 ± 2.16***
HAAR 400mg/kg	69.59 ±2.01*	53.29 ±1.43*	73.96 ± 2.47***	26.43 ± 1.37***
Metformin 300mg/kg	59.02±1.61***	34.680 ± 0.95***	69.12 ± 1.60***	38.29 ± 1.33***

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test; *p<0.05, **p<0.01, ***p<0.00 *vs*. Control Group

Table 8 : Effect Of Multi Dose Of Extract(S) On Body Weight Of Normoglycemic Rats.

Treatment ↓	Body Weight (gm)		Percentage Change
	Day 0	Day 9	
Normal Control	198.33 ± 6.90	210.66 ± 7.18	6.22
HAAR 200mg/kg	203.17±7.59	214.50±7.33	5.58
HAAR 400mg/kg	204.83±4.36	218.67±4.51	6.76
Metformin 300mg/kg	210.00 ± 4.08	177.66 ± 7.17**	- 15.4

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test; *p<0.05, **p<0.01, ***p<0.001 *vs*. Control Group; "-" sign indicates decrease in the value from the initial value

Table 9 : - Effect Of Multi Dose Of Extract(S) On Body Weight Of Hyperglycemic Rats

Treatment↓	Body Weight (gm)		% Change Between
	Day 0	Day 14	Day - 0 & Day - 14
Diabetic Control	203.50±7.33	133.83±5.81	-34.24
HAAR 200mg/kg	200.17±4.64	201.50±1.86***	0.66
HAAR 400mg/kg	200.83±4.03	206.50±4.67***	2.82
Metformin 300mg/kg	202.00±5.47	140.00±4.20	-30.69

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test; *p<0.05, **p<0.01, ***p<0.00 *vs*. Control Group; "-" sign indicates decrease in the value from the initial value

Table 10 Shows: - Effect Of Multi Dose Of Extract(S) On Water Intake Of Hyperglycemic Rats

Treatment↓	Water Intake Habit (ml/rat/day)			% Change Between
	Week 0	Week 1	Week 2	Week - 0 & Week - 2
Diabetic Control	76.83±0.60	89.33±1.17	100.17±1.66	30.38
HAAR 200mg/kg	78.67 ± 0.67	63.16 ±1.91***	48.67 ±2.17***	-38.13
HAAR 400mg/kg	78.83 ±0.79	59.33 ±1.59***	40.17 ±0.79***	-49.04
Metformin 300mg/kg	79.33± 0.95	59.16±1.01***	39.83±1.30***	-49.79

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test; *p<0.05, **p<0.01, ***p<0.00 *vs*. Control Group; "-" sign indicates decrease in the value from the initial value

Table 11 Shows: - Effect Of Multi Dose Of Extract(S) On Food Intake Of Hyperglycemic Rats

Treatment↓	Food Intake Habit (gm/rat/day)			% Change Between
	Week 0	Week 1	Week 2	Week - 0 & Week - 2
Diabetic Control	26.66±0.76	38.33±1.02	41.50±0.96	55.66
HAAR 200mg/kg	28.67 ± 0.71	21.67 ±0.72***	15.33 ±0.67***	-46.53
HAAR 400mg/kg	29.17 ±0.60	19.33 ±0.76***	13.83 ±0.48***	-52.59
Metformin 300mg/kg	27.50±0.76	19.17±0.87***	13.67±0.88***	-50.29

Values are expressed as Mean ± SEM.; (n = 6); One Way ANOVA followed by Turkey – Kramer Multiple Comparison test; *p<0.05, **p<0.01, ***p<0.00 *vs*. Control Group; "-" sign indicates decrease in the value from the initial value

DISCUSSION

The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves over-production of glucose (excessive hepatic glycogenolysis and gluconeogenesis) and/or decreased utilization of glucose by the tissues [10]. The present investigation focus on the efficiency of the hydro-alcoholic leaf extract of *Annona reticulata* in the regulation of blood glucose levels in normal and STZ induced

hyperglycemic rats. When hydro-alcoholic leaf extract of *Annona reticulata* was administered to glucose loaded overnight fasting normal rats, the increase in blood glucose level was observed for 60 min. to reach the maximum level. In case of control group and the metformin treated group, the blood glucose level reaches the maximum level 30 minutes after the oral glucose loading. The observations of Oral Glucose challenge test showed that; pretreatment with the extract delays the duration of increase in the blood glucose level to reach a maximum level when compared with control and standard treated group. This indicates that the extract may be providing resistance to intestinal glucose absorption to certain extent. Then decrease in blood glucose from maximum level may be attributed to pancreatic or expancreatic effect of the extract or increased utilization of glucose by the tissues. Administration of hydro-alcoholic extract of *Annona reticulata* to diabetic rats showed a significant decrease in the levels of blood glucose. The possible mechanism by which *Annona reticulata* brings about its hypoglycemic action in diabetic rat may be due to increase in insulin level in plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form. There is marked increase in serum triglycerides and cholesterol observed in untreated diabetic rats. Elevation of plasma lipid concentration in diabetes is well documented. Under normal circumstances insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzymes thereby causing hypertriglyceridemia and hypercholesterolemia [11]. The significant control of the levels of serum lipids in the hydro-alcoholic extract treated diabetic rats may be attributed to improvements in insulin levels upon *Annona reticulate* therapy. In insulin deficient diabetics, the plasma free fatty acid concentration is elevated as a result of increased free fatty acid outflow from fat depots, where the balance of the free fatty acid esterification–triglyceride lipolysis cycle is displaced in favour of lipolysis. It is well known that streptozotocin-provoked hyperglycemia accompanied by symptoms like loss of weight, polydipsia and polyphagia [12]. Induction of diabetes with STZ is also associated with the characteristic loss of body weight which is due to increased muscle wasting in diabetes [13]. In our study, the extract not only significantly prevented loss in body weight in diabetic rats but also diminished food intake and the water consumption when compared with diabetic control rats. Diabetic rats treated with the hydro-alcoholic extract showed an increase in body weight as compared to the diabetic control which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis. The improvements of these parameters could be attributed to the hypoglycemic properties of the plant. From the results of the studies done on normal rats it's found that, hydro-alcoholic extract of *Annona reticulata* has very minimal effect on blood glucose level of normal rats even when administered in multiple doses. So, it may be postulated from the experimental results that; the extract doesn't have any hypoglycemic effect. Administration of hydro-alcoholic extract to normal rats did not significantly alter body weight and plasma glucose during the 9-day period. Oxidative stress has been shown to play a role in the causation of diabetes I and II and as such, antioxidants may have a role in the alleviation of diabetes [14]. STZ produces oxygen radicals in the body, which cause pancreatic injury and could be responsible for increased blood sugar seen in animals [15]. Studies suggest that *Annona reticulata* leaves possess potent antioxidant activity [16]. This may be due to the presence of phenols and flavonoids which may have a major role in reducing oxidative stress associated with diabetes. Thus the observed antidiabetic activity of hydro-alcoholic extract of *Annona reticulata* in our study may be attributed to the antioxidant property of the plant as well. The antidiabetic activity was not found to be dose dependant as there was no significant difference between the 200 mg/kg and 400 mg/kg extract treated groups.

CONCLUSION

The results of the present study suggest that *Annona reticulata* leaves possess potent glucose lowering effect. The glucose lowering activity is more of corrective in nature than disruptive. Longer duration studies of *Annona reticulata* and its isolated compounds on chronic models are necessary to understand the exact mode of action and develop a potent antidiabetic drug.

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Declaration of interest

The authors declare that they have no conflicts of interest to disclose.

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