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ANTI- HYPERLIPIDEMIC AND ANTI-DIABETIC ACTIVITY OF METHANOLIC EXTRACT OF *ANNONA RETICULTA* IN ALBINO RATS.

Prabin Mallick[1], Jimidi Bhaskar*[2]

[1]Department of Pharmacology, Bharat Institute of Pharmacy, Mangalpally, Ibrahimpatnam, Ranga Reddy, Telanga – 501510.

[2]Department of Pharmaceutics, Bharat Institute of Pharmacy, Mangalpally, Ibrahimpatnam, Ranga Reddy, Telanga – 501510.

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***Corresponding Author: Dr. Jimidi Bhaskar**
Department of Pharmaceutics, Bharat Institute of Pharmacy, Mangalpally, Ibrahimpatnam, Ranga Reddy, Telanga – 501510.
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ABSTRACT

The present study was designed to assess the anti - hyperlipidaemic and anti - diabetic Activity of methanolic extract of *Annona Reticulta* in Albino Rats. The plant extracts of *Annona Reticulta* were administered orally with maximum dose of 2000 mg/kg bodyweight The biochemical estimations were done by using specific procedures. Cholesterol, Triglycerides, HDL-Cholesterol, SGOT/AST, SGPT/ ALT, Alkaline Phosphatase, Serum Total Proteins and Glucose were determined. The extract also show increase in the glucose tolerance of the rats and decrease in the fasting blood glucoselevel of diabetic rats, showing the hypoglycaemic activity of the plant which is most pronounced in methanol extract. The results of this investigation showed that rats treated with a methanol extract of *Annona Reticulta* leaves have both anti -hyperlipidaemic and anti-diabetic activity.

KEYWORDS: *Annona Reticulta*, Leaves, anti- hyperlipidaemic, Rat.

INTRODUCTION

Traditional medicine is looked upon as an alternative or supplement to modern medicine and has made significant contributions to the health care of the world over the past decades. Various diseases such as diarrhoea, skin problems, headache, fever, cough, wounds, hypertension, diabetes.[1] and rheumatism are treated with herbal medicine. Traditional medicines continue to be practiced by the community to treat disease and maintain health especially in remote areas where modern facilities are not readily available. Most of the medicinal plant species are collected from the wild, a few are being cultivated. Diabetes mellitus is a metabolic disorder in the endocrine system. This dreadful disease is found in all parts of the world and is becoming a serious threat to mankind health. There are lots of chemical agents available to

control and to treat diabetic patients but total recovery from diabetes has not been reported up to this date. Alternative to these synthetic agents, plants provide a potential source.[2,3] of hypoglycaemic drugs and are widely used in several traditional systems of medicine to prevent diabetes. Herbal therapy for diabetes has been followed all over the world successfully. Herbs were used to manage Type 1 and Type II diabetes and their complications.[4] The herbs have been considered for their possible hypoglycaemic actions and the researchers have carried out some preliminary investigations. Hence the present study was designed to assess the anti-hyperlipidemic and anti - diabetic Activity of methanolic extract of *Annona Reticulta* in Albino Rats.

MATERIALS ANDMETHODS

Plant Material

The leaves of plant *Annona Reticulta* was collected from hilly region of chittoor district, Tirupathi, A.P, India. The plant was authenticated by Dr. K. Madhav Chetty, Asst. Professor, Dept. of Botany, Sri Venkateshwara University, Tirupathi.

Experimental Animals

Male Wistar rats weighing (180-220g) were provided by animal house of Sigma Institute of Clinical Research and Administration (SICRA Labs), Kukatpally, Hyderabad, India. They were housed in ventilated rooms at a temperature of 24±2^{o}c with a 12h light/dark cycle and 54±5% relative humidity, maintained on standard pellet and water ad libitum throughout the experimental period. The animals were acclimatized for a period of one week. The experiments were carried out according to the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethical Committee (IAEC) of Sigma Institute of Clinical Research and Administration pvt.ltd. Hyderabad.

Preparation of Extract

The collected plant was shade dried for 4 weeks and was ground to course powder using mixer grinder. The powdered plant material leaf (250gm) was extracted with methanol, by Maceration process. Finally extracts were air dried at room temperature. 10.2% and 7.2% w/w extract thus obtained was subjected for evaluation of Hypoglycemic activity in alloxan induced diabetic rats. The test samples of extracts were made in appropriate concentrations using distilled water prior to its use for animal studies.

Phytochemical Screening.[5]

Preliminary phytochemical investigation was carried out on Methanol extract of *Annona Reticulta* leaf for detection of various phytochemicals by standard methods.

Acute oral toxicity

Acute toxicity studies were performed according to OECD-423 guidelines category IV substance (acute toxic class method). Albino rats (n=3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 hrs with free access to water only. The plant extracts of *Annona Reticulta* were administered orally with maximum dose of 2000 mg/kg body weight. The mortality was observed for three days. If mortality was observed in 2/3 or 3/3 of animals, then the dose administered was considered as a toxic dose. However, if the mortality was observed only one rat out of three animals, then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher dose (Organization for economic Co-operation and development, 2001).

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test (OGTT) measures the body's ability to use a type of sugar called glucose, that is the body's main source of energy, OGTT, a test of immense value and sentiment, in favour of using fasting plasma glucose concentration alone was seen as a practical attempt to simplify and facilitate the diagnosis of diabetes. Hyperglycemia is an important factor in the development and progress of the complications of diabetes mellitus.

Oral glucose tolerance test on Diabetic rats (OGTT)

Fasting blood glucose was determined after depriving food for 16 h with free access of drinking water. Hyperglycemia was induced by a single I.P. injection of 120 mg/kg of alloxan monohydrate (Avra synthesis. Ltd., Hyderabad, India) in sterile saline. After 2 days of alloxan injection, the hyperglycemic rats (glucose level > 200mg/dl) were separated and divided into different groups comprising of 6 rats each for the hypoglycemic study.

The overnight fasted rats of all the groups were loaded with glucose (2gm/kg P.O) 30 minutes after drug administration. Blood samples were collected from the tail vein puncture method prior to drug administration and at 0, 30, 60, 90, minute after glucose loading. Serum glucose levels were measured immediately. The glucose level was estimated using digital glucometer Six fasted animals were used in each group.

Rats were divided into following groups.

Group- I – Received Vehicle- Distilled water (Control –Ve) Group-II – Received 2 gm /kg glucose P.O. (control +Ve)

Group III - Received standard drug Metformin (150 mg/kg), P.O.

Group IV – Received methanolic extract of leaf of Barleria longiflora, dose 400 mg/kg, P.O. Group V – Received extract of the plant leaf of *Annona Reticulta*, dose 400mg/kg, P.O.

Biochemical parameters[6-8]

Experimental Animals

Wistar albino adult male rats weighing 200-250g were obtained from the animal house. The animal were grouped and housed in polyacrylic cages (38x 23x 10 cm) with not more than five animals per cage and maintained under standard laboratory under standard laboratory conditions (temperature 25+2oC) with dark and light cycle (14/10 hour). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The mice were acclimatized to laboratory condition for 10 days before commencement of experiment. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA. High Fat Diet was given to experimental animals followed by test.

Induction of Hyperlipidemia by Triton-x-100

Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 hours.

The animals were divided into five groups of six rats each.

I. The first group was given standard pellet diet, water and orally administered with 2% Tween 80.
II. The second group was given a single dose of triton administered at a dose of 100mg/kg, I.P. After 72 hours of triton injection, this group received a daily dose of 2% Tween 80 (P.O) for 7 days.
III. The third group was administered a daily dose of Atorvastatin 10 mg/day
IV. Fourth group *Annona Reticulta* 200mg/kg suspended in 2% Tween 80, P.O, for 7 days, after inducing hyperlipidemia.

V. Fifth group was administered with the *Annona Reticulta* 400 mg/kg, P.O. for 7 days.

Induction of Hyperlipidemia by High Fat Diet

The animals were divided into five groups. Each group contains six animals. Grouping is as follows:

Group 1: Normal Group (Tween 80 Group 2: Control Group (HFD)

Group 3: Standard-Atorvastatin + HFD (10 mg/kg)

Group 4: Extract II- *Annona Reticulta* + HFD (400 mg/kg) Group 5: Extract I- *Annona Reticulta* + HFD (200 mg/kg)

The study Duration is 14 days

Collection of blood

On the 8thday, blood was collected by retro orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and used for various biochemical experiments. The animals were then sacrificed and the liver collected.

Bio Chemical Estimations

The biochemical estimations were done by using specific procedures. Cholesterol, Triglycerides, HDL-Cholesterol, SGOT/AST, SGPT/ ALT, Alkaline Phosphatase, Serum Total Proteins and Glucose were determined.

Statistical Analysis

All data were expressed as the mean ± SEM. For statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) followed by Dunnett's test, $P<0.05$ was considered significant

RESULTS AND ISCUSSION

Phytochemical Analysis

The results of the preliminary phytochemical screening of methanolic extracts of ***Annona Reticulta*** as summarized in Table 1 revealed the presence of various substances.

Table 1: Preliminary phytochemical screening of *Annona Reticulta* extract.

Substances	Test	Results
Alkaloid	Mayer's test	+
Carbohydrates	Molisch's test	+
Glycosides	Legal test	+
Saponins	Foam test	+

Determination of Acute Oral Toxicity of EBG

The plant leaf extract of *Annona Reticulta* didn't show any mortality and toxicity even at highest dose of 2000 mg/kg body weight employed. The present research study was carried out using dose (400mg/kg body weight) for Hypoglycemic activity It was observed that the no toxic effect after administration of MBG like no tremor, no convulsions, no salivation, no diarrhoea, and no lethargy.

OGTT on Diabetic rats

Methanol extract of Annona Reticulta leaf (400 mg/kg) significantly decreased blood glucose level in glucose fed rats at 90 minutes when compared with the control group. It also decreased the elevated blood glucose at 60 minutes after the glucose administration methanol extract of the control group showed significant increase in blood glucose level when compared with the normal group. Metformin showed it's potent anti-diabetic activity at 90 minutes. Also the

reduction in elevated blood glucose level at 30 and 60 minutes after the administration of glucose was significant when compared to the control group. These data suggested that treatment with methanol extract of Annona Reticulta leaf showed better tolerance to exogenously administered glucose. The effect of methanol extract of *Annona Reticulta* on glucose tolerance test in Diabetic fasted rats is shown in Table No.

Table no. 11: Effect of methanol extract of Annona Reticulta leaf on oral glucose tolerance Test.

GROUPS	Blood glucose level mg/dl			
	0 min	30 min	60 min	90 min
Normal	95.69 ± 1.51	85.95 ± 1.37	93.83 ± 0.94	94.51 ± 0.99
Control (ALX 120mg/kg)	264.84 ± 1.26[a]	277.01 ± 1.19[a]	269.86 ± 1.01[a]	260.18 ± 1.12[a]
Standard (MET 150mg/kg)	275.45 ± 1.52	203.01 ± 1.39***	159.72 ± 1.05***	108.39 ± 1.04***
Annona Reticulta methanol (200mg/kg)	273.02 ± 1.43	217.67 ± 1.60***	197.94 ± 1.22***	104.64 ± 0.89***
Annona Reticulta methanol 400 mg/kg)	283.03 ± 1.21	213.49 ± 1.34***	178.02 ± 1.18***	106.15 ± 1.03***

All the values are expressed as Mean ± SEM, n=6, One way analysis of variance followed by multiple comparison Dunnet's test, *P< 0.05, **P<0.01 and ***P<0.001 as compared to control and [a]P<0.001, [b]P<0.01 and [c]P<0.001 when compared to normal group.

Evaluation of Anti Hyperlipidaemic activity of *Annona Reticulta* In Rats Table 12 Triton-X-100 Induced Model:

Triton x-100					
TEST	**NORMAL**	**CONTROL**	**STANDARD**	**T1**	**T2**
ALP	76.85±2.707	180.41±2.23***	113.83±1.54***	135.76±0.81***	82.3±1.573
GPT	34.34±2.286	57.90±2.504***	37.08±2.741	50.79±1.385***	45.83±2.893**
GOT	42.49±4.388	54.80±2.975*	42.78±3.262	41.27±1.520	45.12±3.061
TP	39.41±2.245	22.69±0.864***	16.40±1.204***	15.17±1.114***	17.99±1.229***
HDL	52.64±2.520	22.58±2.209***	42.52±1.893**	50.14±1.233	55.85±1.163
TG	50.08±1.180	77.97±1.553***	69.27±1.758***	77.3±1.790***	66.54±1.233***
TC	74.22±1.401	136.04±3.954***	67.07±1.840	99.44±2.785***	91.14±2.631***
VLDL	10.01±0.233	15.59±0.627***	13.85±0.352***	15.45±0.356***	13.30±1.464***
LDL	11.56±2.692	101.63±5.069***	10.63±3.114	33.83±4.159	21.98±2.700
AI	0.42±0.537	6.48±0.620	0.58±0.529	0.99±0.604	0.63±0.540
CRR	1.42±0.589	7.48±0.721	1.58±0.529	1.99±0.604	1.63±0.540

Table 13: Body Weight.

TRITON X100	NORMAL	CONTROL	STANDARD	T1	T2
Before treatment	173.0±0.96	172.66±0.89	174.0±1.12	173.33±0.88	172.0±0.85
After treatment	183.5±0.76	243.0±0.96***	194.33±0.66***	224.33±0.88***	244.83±1.14***

N = 6; Significance: *** *P*<0.001, ** *P*<0.01, * *P*<0.05 from control

HFD Diet Induced Model

Table no. 14: Biochemical Parameters of the Animals.

HFD diet	NORMAL	CONTROL	STANDARD	T1	T2
B.w B.T	228.83±0.8	228±1.07	227.83±1	227.5±0.71	226.16±0.91
B.w A.T	262.5±0.77	311.83±1.6***	281.33±0.9***	293.83±1.27***	290±1.19***
HDL	27.00±0.81	22.7±0.71**	31.55±0.54**	31.04±0.85**	37.98±0.76***
LDL	25.20±0.82	61.88±0.95***	35.60±0.78***	41.31±1.11***	24.81±1.09
VLDL	11.32±0.68	16.11±0.85***	12.31±0.58	14.32±0.39***	12.37±0.58
GLUCOSE	73.51±0.90	146.4±0.85***	104.2±0.91***	121.2±0.7***	106.2±0.72***
TC	65.03±0.97	106.0±0.63***	84.7±0.80***	83.49±1.17***	67.36±0.57
TG	54.08±1.35	94.04±0.68***	63.94±1.16***	73.41±1.31***	54.91±0.93
AI	1.41±0.574	3.67±0.529***	1.66±0.619	1.69±0.46	0.77±0.98***
CRR	2.41±0.574	4.67±0.529***	2.68±0.528	2.69±0.46	1.77±0.98***

CONCLUSION

In the present study an increase in plasma HDL-cholesterol with a concomitant percentage decrease from others lipid was observed. It can be concluded from the present data that the levels of total serum cholesterol, triglyceride and MDA which are actually raised in high fat diet, can be lowered significantly with Annona Reticulta and total proteins which is actually lowered in Triton-x-100 can be raised significantly with *Annona Reticulta***.** Atherogenic index which actually raised in atherogenic diet and Triton-x-100, can be lowered significantly with Annona Reticulta and a very good % protection was seen with *Annona Reticulta* and standard drug. The extract also show increase in the glucose tolerance of the rats and decrease in the fasting blood glucose level of diabetic rats, showing the hypoglycaemic activity of the plant which is most pronounced in methanol extract. In nutshell the extract of *Annona Reticulta* possesses significant hypoglycaemic activity and anti - Hyperlipidaemic activity, which is the first claim in this respect.

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