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ANTI-DIABETIC ACTIVITY AND ANTI-HYPERLIPIDEMIC ACTIVITY OF BARLERIA LONGIFLORA

KUTHATI THIRUPATHAMA[1]*, K. CHAITANYA PRASAD[2], B. SUDHAKAR[3]
[1]DEPARTMENT OF PHARMACOLOGY, SAMSKRUTI COLLEGE OF PHARMACY IN GHATKESAR, TELANGANA, 501301.

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Abstract:

Objective: *- To investigate the anti diabetic and anti Hyperlipidemic activity of methanol extract of Barleria longiflora in male Wistar rats.*

Material & method: *- In this model of Hyperlipidemia, 30 adult male wistar rats (150-200gms) were evenly divided into 5 groups in both groups. Group-1 and Group-2 served as untreated and model controls respectively, while Group-3, 4 and 5 were the treatments groups which were simultaneously treated with standard, 200 and 400 mg/kg extract respectively along with High Fat Diet and Triton x 100. On last day, blood samples for biochemical parameters, were obtained under inhaled diether anaesthesia.*

In the model of anti diabetic animals were evenly divided into 5 groups

Group-1 and Group-2 served as untreated and model controls respectively, while Group-3, 4 and 5 were the treatments groups which were simultaneously treated with standard, 200 and 400 mg/kg extract respectively after glucose loading.

Results: *- HFD and Triton x 100 treatment caused Hyperlipidemia as evidenced by marked elevation in Cholesterol, Triglycerides, LDL, VLDL and decrease in HDL levels. Co-administration of extract with HFD and Triton x 100 decreased rise Cholesterol, Triglycerides, LDL, VLDL and increase in HDL levels.*

Glucose loading caused hyperglycemia by elevation of glucose which was significantly reduced by treatment with standard and extract.

Conclusion: *It was observed that the methanol extract of Barleria longiflora conferred anti diabetic and Anti- Hyperlipidemia activity by biochemical observation against HFD and Triton-x-100 induced Hyperlipidemia in rats. In the near future could constitute a lead to discovery of a novel drug for treatment of drug induced Hyperlipidemia and diabetes.*

Corresponding author:
Kuthati Thirupathama,
Department of Pharmacology, Samskruti College of Pharmacy, Ghatkesar, Telangana. Email Id- kuthatithiru@gmail.com

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INTRODUCTION:

Diabetes Mellitus:

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 diarrhea, skin problems, headache, fever, cough, wounds, hypertension, diabetes 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. [1]

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 of hypoglycemic drugs and are widely used in several traditional systems of medicine to prevent diabetes. [2]

Introduction to diabetes mellitus:

Diabetes Mellitus is a disease caused by the failure of the body function properly carbohydrate metabolism as well as changes in lipid and protein metabolism, thus contributing to hyperglycemia (increased blood sugar levels above normal), glycosuria (the presence of sugar in the urine), polyuria (the need to urinate frequently), polydipsia (always thirsty) and polyphagia (increase appetite). Hyperglycaemia caused by the failure of the pancreas secrete insulin insatiety, insulin resistance and the reduction of glucose by cells[19]. Diabetes is the category of chronic diseases which is still no cure and cause affliction in the patient. The increasing in cases of diabetes around the world becomes a major problem that must be addressed by society[20]. Global population worldwide are at high risk of diabetes and in America about 15.6 million people have been diagnosed with diabetes [21].

History of Diabetes Mellitus:

The term “diabetes” is derived from the Greek word which means “excessive urine production”, while the term "Mellitus" comes from Latin which means "sweet". Thus, the continuous increase of sugar in the blood is excreted through the urine and causes urine and blood on the sweetness of individuals with diabetes [19].

Classification of Diabetes Mellitus:

World Health Organization (WHO) classified diabetes mellitus into three types based on the etiology Diabetes Type I (Insulin-Dependent Diabetes Mellitus: IDDM), Diabetes Type II (Non-Insulin-Dependent Diabetes Mellitus: NIDDM) and Gestational Diabetes. Diabetes Type I caused by the failure of the pancreas produce insulin and without insulin the liver is unable to regulate blood glucose levels properly. Type I diabetes usually occurs in children aged between 9-14 years and adolescents. Diabetes Type II is caused by a reduction in tissue sensitivity to insulin or resistance to insulin and it usually occurs in adults. Individuals who are obese face a higher risk of developing Diabetes Type II compared with normal individuals, while gestational diabetes occurs during pregnancy [19].

Pathogenesis of Diabetes Mellitus:

Diabetes Type I:

i. Genetic factor:

Genetic factors play a role in the development of Diabetes Type I, where more than one gene involved. Diabetes Type I is related with Histocompatibility Locus Antigen (HLA) and the study findings show higher HLA antigens in patients with diabetes compared with normal individuals. Genes involved in the discovery of HLA antigen found on the chromosome-6 and found more than one gene involved in the determination of HLA antigens such as B8 and B3. HLA antigen is a type of glycoprotein present in all human cell membranes and is believed responsible for detecting and destroying foreign molecule like bacteria and viruses. However, individuals with HLA antigens associated with diabetes do not guarantee the holder will have diabetes, but the risk of diabetes is higher[22].

ii. Autoimmune reaction:

Type 1 diabetes mellitus (T1DM) is the result of immune-mediated destruction of insulin-secreting pancreatic beta cells. T cells that react to islet beta cells can contribute to the autoimmune response in diabetic patients and also play a part in self-tolerance in healthy individuals. The hormone insulin is usually not available directly in the diabetes type I. Changes in the function of HLA antigens normal detected as a factorcontributing to the autoimmune reaction resulting in the destruction of his own cells including the cells causes cells could not recognize its own beta cells of the host cell and more seriously, it is known to foreign molecules, further stimulate the production of antibodies to attack the beta cells causes insulin can not be produced [22].

Diabetes Type II:

i. Genetic factor:

Diabetes Type II is influence by genetic factors which is high risk individuals if they have parents who suffer from diabetes type II. However, environment and lifestyle also as important role in the development of diabetes type II. Unhealthy lifestyle causes obesity increases the risk of developing diabetes[22].

ii. Insulin resistant:

Insulin resistant characterized by reduced responsiveness to normal circulating concentrations of insulin is a common feature of almost all patients with type II diabetes. The presumed central roles of both peripheral and hepatic insulin resistance suggest that the enhancement of insulin action might be an effective pharmacological approach to diabetes. The severity depends on the degree of hyperglycemia and insulin resistance, it occurs in the liver tissue and muscle tissue caused by changes in insulin receptor found on the surface of the liver tissue and muscle tissue. Insulin resistance in the liver causing excessive glucose production and glucose consumption low during full [23].

Gestational diabetes is carbohydrate intolerance resulting in hyperglycemia of variable severity with onset or first recognition during pregnancy. It does not exclude the possibility that the glucose intolerance may antedate pregnancy but has been previously unrecognized. The definition applies irrespective of whether or not insulin is used for treatment or the condition persists after pregnancy. Problem of resistance to insulin causes insulin receptors expressed less then give effect to the dispatch system signals [19].

Complication of Diabetes Mellitus:

Diabetes is a complex condition which can result in long term complications. There is no such thing as „mild" diabetes. Whether diabetes is managed by healthy eating and physical activity alone or in conjunction with tablets and or injections, poorly controlled diabetes will cause damage to your body. High blood glucose levels over a period of time can damage the small and large blood vessels and nerves. The most common complications that occur in people with diabetes include:

- Cardiovascular disease contributed to death of adult diabetes in which the frequency of heart attacks for individuals with diabetes are three times higher than normal individuals [24].
- Diabetic neuropathy is a complication of diabetes, where almost 50% of patients with diabetic neuropathy diabetic disease resulting from continued improvements in blood sugar. High risk feet have lost feeling (peripheral neuropathy) and poor blood flow (peripheral vascular disease). People who have had a foot ulcer or amputation in the past have high risk feet. Feet with calluses or deformities like claw toes also have increased risk if poor feeling and or decreased blood flow are also present [24].
- Diabetic rethinopathy causes a reduction in the sense of sight in about 95% of patients with type II diabetes caused by obstructions in blood vessels of the retina is almost 2 % of patients with diabetes become blind [25].
- Diabetes causes kidney function, the study showed that about 10% of patients with diabetes experience kidney failure [26].

MATERIALS AND METHODS:

Plat material:

The leaves of plant *Barleria longiflora* 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°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.

Drugs and chemicals:

Metformin, Alloxan monohydrate, all other chemicals and diagnostic kits were provided by Sigma Institute of Clinical Research and Administration.

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[93]. 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:
Preliminary phytochemical investigation was carried out on Methanol extract of *Barleria longiflora* leaf for detection of various phytochemical by standard methods

Determination of 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 ***Barleria longiflora*** 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 favor 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 drinkin g 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/kgp.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 *Barleria longiflora*, dose 400mg/kg,p.o.

The parameters studied were as follows:
Biochemical parameters such as:
Blood Glucose

I. Chemicals

Triton X-100(a non-ionic detergent, iso octyl polyoxy ethylene phenol, formaldehyde polymer) was obtained from Technicho lab chemicals, Coimbatore. Atorvastatin was obtained from Moral labs, Chennai. All other chemicals were of analytical grade and obtained locally.

II. High Fat Diet Composition:

Composition	Normal diet (%)	High Fat diet (%)
Protein(Milk powder)	12	10
Carbohydrates (Wheat flour)	71	61
Sugar	05	05
Fat (Butter)	05	16
Salts	04	04

Vitamins	01	02
Fibers	02	01
Cholesterol	--	01
Total Weight	100g	100 g

III. 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.

IV. 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 h95.

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 *Barleria longiflora* 200mg/kg suspended in 2% Tween 80, p.o., for 7 days, after inducing hyperlipidemia.
V. Fifth group was administered with the *Barleria longiflora* 400 mg/kg, p.o. for 7 days.

RESULTS:

List of Instruments used

Sr.No.	Name of Instrument	Description
1.	Autoanalyser	ARTOS, The versatile Autoanalyser, Sl No-SBPL/ 188/06-07
2.	Incubator	REMI Cooling Centrifuge. C-24 BL.
3.	Digital Balance	ACCULAB – Sartorious group.
4.	Flash Evaporator	SUPERFIT, Rotary “Vaccum Digital Bath”, PMTc – 3040
5.	Deep Freezer	BLUE STAR, Model No.- CHE 400, Sr No.-67771
6.	Homogenizer	REMI Homogenizer Mumbai. Type – RQ 127A

Table List of Chemicals used

Sr.No	Name of Chemical	Description
1.	Gentamicin	Gifted by Microlabs Pvt. Ltd. Bangalore
2.	Heparin	(HEP-5) Gland Pharma Ltd, Hyderabad. Batch – No. UJ918
3.	Chloroform	S.D. Fine – Chem Ltd. Mumbai.
4.	KCl	S.D. Fine– Chem Ltd. Mumbai. Batch-No: -200Z-0200-1612-09. Mole. Wt:- 74.55
5.	Formalin	Fischer Scientific. Lot No:- 91026906-5., Pdt No.24005
6.	NaCl	LeoChem, Bangalore. Mole.Wt:- 58.44, Lot No. 126012
7.	EDTA	S.D.Fine Chem Ltd. Pdt No:- LO4/10204/2611/13
8.	KH_2PO_4	Qualigens fine Chemicals, Mumbai. Lot No:- 18986711-
9.	K_2HPO_4	Leochem, Bangalore. Lot No- 125176, P-2V829.
10.	H_2O_2 (30%w/v)	SDFCL-38694L05,Batch No-G09A/2209/0807/13
11.	Methanol	SDFCL. Mole Wt:- 32.04, B.P. 64-65.5 ºC, Batch No:- K08A/1308/1211/13
12.	Trichloroacetic acid	Nice Chemicals, Bombay.
13.	Thiobarbituric acid (TBA)	Loba Chemicals, Mumbai.
14.	Sodium azide	S.D. Fine Chem Ltd.
15.	Reduced glutathione	Sigma U.S.A.
19.	Urea Kit	Coral Clinical Systems, Verna Goa, India.
20.	Uric acid Kit	Coral Clinical Systems, Verna Goa, India.
21.	Creatinine Kit	Coral Clinical Systems, Verna Goa, India.

Determination of Acute Oral Toxicity of EBG:

The plant leaf extract of *Barleria longiflora* didn't shown 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

Table no 10: Toxicity record sheet: The toxicity record sheet is as follows:

S.no.	Code	Toxicity		Time Of Dea	Observation										
		Onse	Stop		Skin colour	Eyes	Resp	CNS	Tre	Con	Sali	Diah	Sleep	Leth	
1.	MBG	x	X	x	x	X	x	X	x	x	x	x	X	x	

(TRE-Tremor, CON-Convulsions, SALI- Salivation, Diah - Diarrhea, LET-Lethargy)

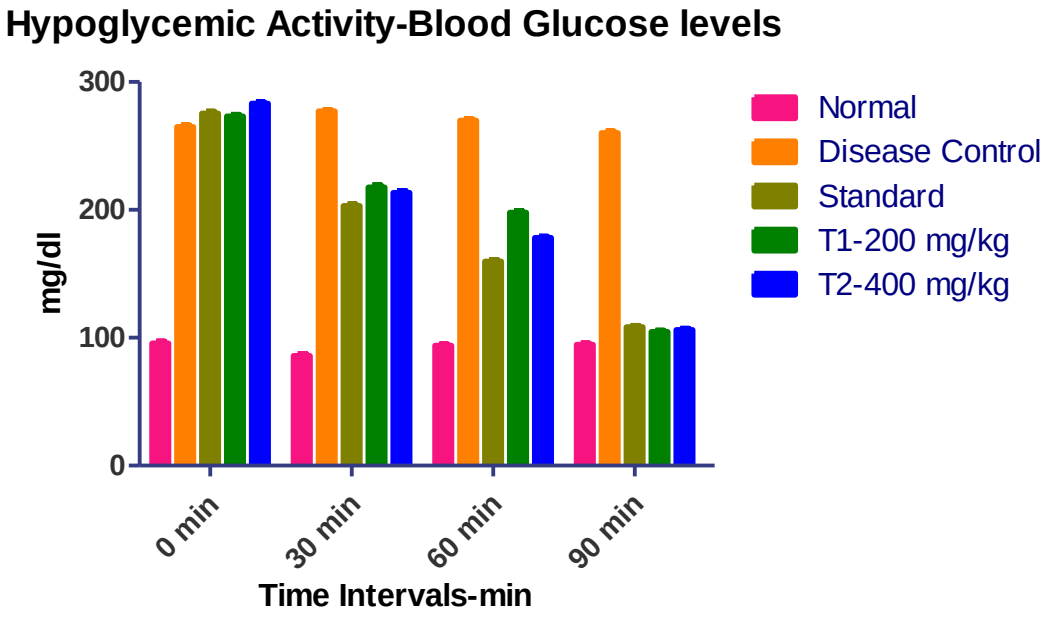
× = Negetive Ø = Positive

OGTT on Diabetic rats:
The effects of methanol extract of Barleria longiflora on glucose tolerance test in Diabetic fasted rats are shown in Table No.

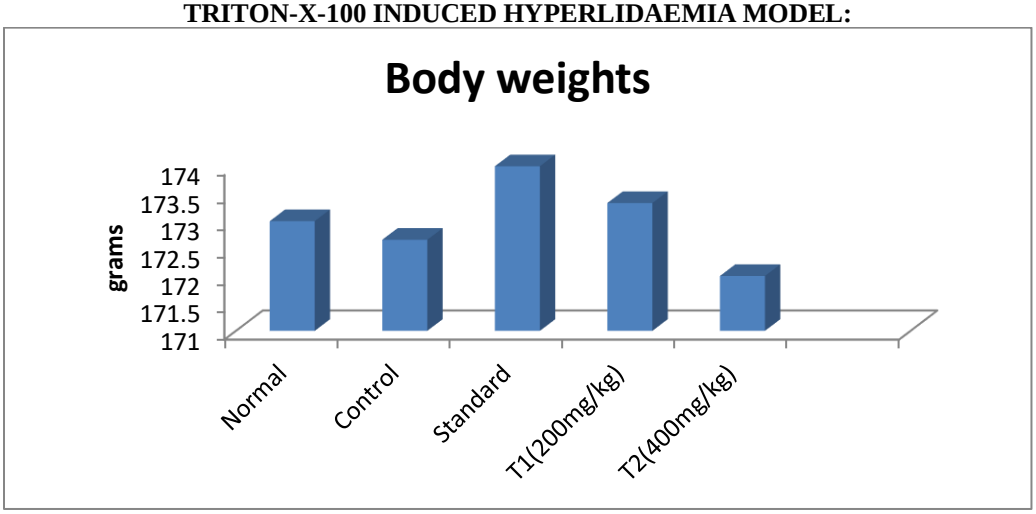
Methanol extract of Barleria longiflora leaf (400 mg/kg) significantly decreased blood glucose level in glucose fed rats at 90 minutes when compared with the control group. It also decreased the elevated blood glucose at 60 minutes after the glucose administration. Methanol extract of the control group showed significant increase in blood glucose level when compared with the normal group.

Metformin showed its 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 *Barleria longiflora* leaf showed better tolerance to exogenously administered glucose.

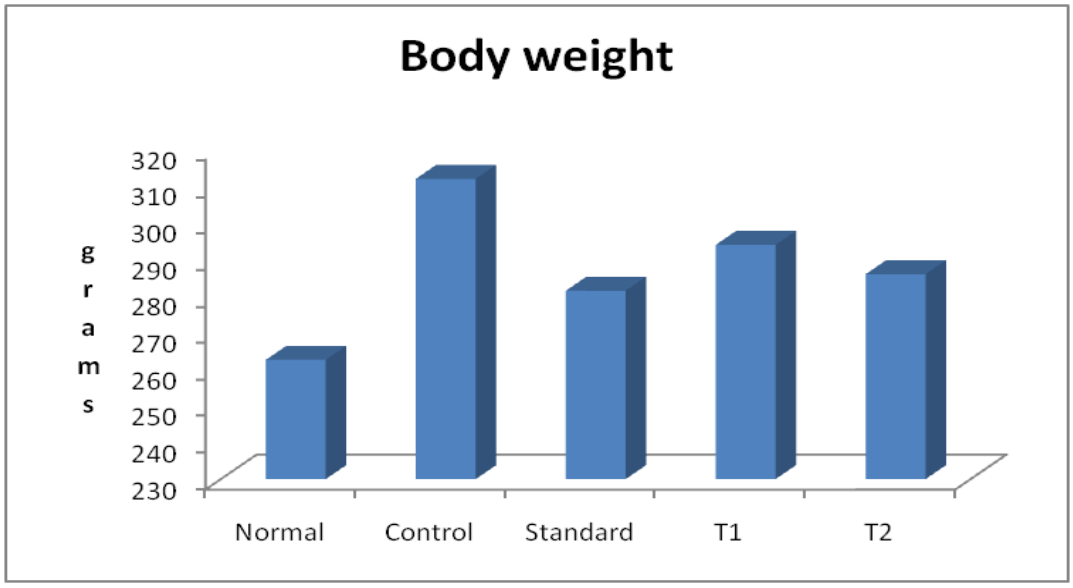


TRITON-X-100 INDUCED HYPERLIDAEMIA MODEL:



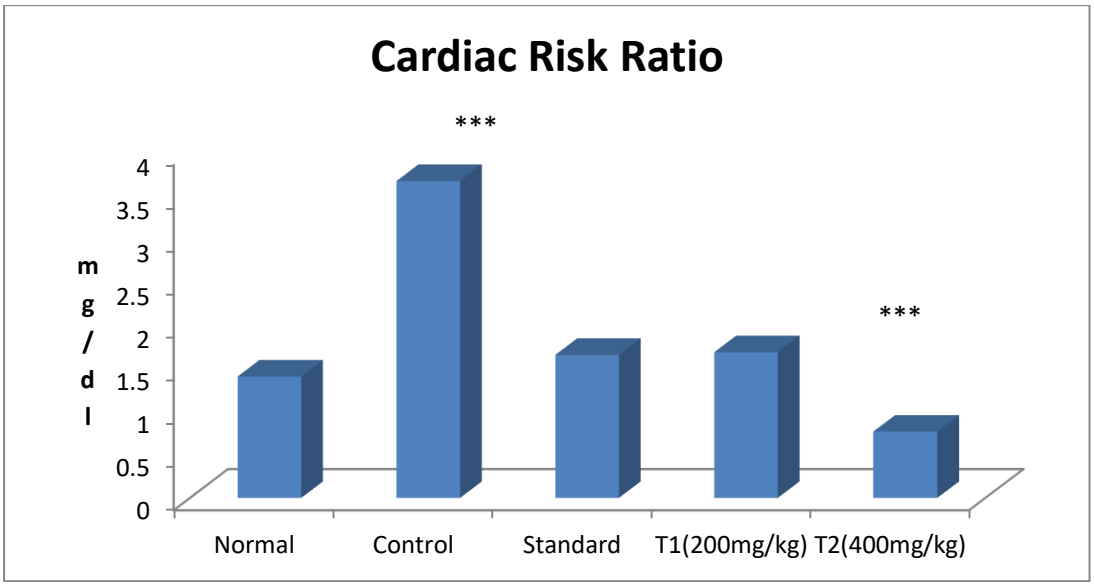
Graph 2 Histogram showing Initial BODY WEIGHT of animals

HIGH FAT DIET MODEL:



Graph 15 Histogram showing the Intial Body weight of animals

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



Graph .24 Histogram showing the effect of *Barleria longiflora* on Cardiac Risk Ratio of animals

DISCUSSION:

The result of this study showed that oral administration Methanol extract of *Barleria longiflora* leaf had a beneficial effect on the diabetic state by reducing hyperglycemia. The extract at doses of 400mg/kg body weight caused a statistically significant (P<0.05) reduction in blood glucose in alloxan induced diabetic rats hypoglycemic study. Alloxan is an oxidation product of uric acid which is widely used for pharmacological induction of diabetes and more useful in multiple aspects of the disease.

From the results of clinical studies, it is evident without any doubt that the reduction of hyperglycemia is the most important factor in the prevention of chronic microvascular complications of diabetes mellitus such as retinopathy, nephropathy, neuropathy, diabetic foot and poor wound healing as well as in the prevention of accelerated

atherosclerosis-related condition (myocardial infarction, stroke, etc.).

The exact mechanism involved in the hypoglycaemic action is not clear. The extract may stimulate insulin secretion by the pancreas or/and enhance insulin sensitivity in various organs especially the muscles by promoting glucose uptake and metabolism inhibiting hepatic gluconeogenesis.

Phytochemical screening of methanol extract of the leaf and part of *Barleria longiflora* revealed the presence of flavonoids, alkaloids, tannins, Flavonoids have been shown to exert their antioxidant activity by various mechanisms by scavenging or quenching free radicals or by inhibiting enzymatic systems responsible for free radical generation.
Apart from being antioxidants, flavonoids have been reported to inhibit sodium-dependent vitamin C transporter 1 and glucose transport Isoform 2 (Glut 2), the intestinal transporter for vitamin C and glucose, leading to a decrease in the intestinal absorption of glucose, hence decrease in the blood glucose concentration. Several researches have also demonstrated that flavonoids act as reducer of hyperglycemia by causing inhibition of renal glucose re absorption through inhibition of the sodium-glucose symporters located in the proximal renal convulated tubule.
Previous studies have reported some of these phytocomponents to elicit a wide range of biological activities which include hypoglycemic, hypolipidemia among others. Specifically, saponin is known to elicit serum cholesterol lowering activity by causing resin-like action, thereby reducing the enterohepatic circulation of bile acids. In the process, the conversion of cholesterol to bile acid is enhanced in the liver resulting in concomitant hypocholesterolemia.

Equally literature has reported the hypoglycemic and hypolipidemic effects of flavonoids, alkaloids and tannins. The presence of these phytocomponentsin the extract in high concentrations could account for these observed biological effects, particularly hypoglycemic and hypolipidemic effects. The mechanism by which the extract exert the hypoglycemic effect may appear to be related to presence of flavonoid among other secondary metabolites or bioactive chemical constituents found in the plant extract which may be an active constituents in a group or an individual responsible for the hypoglycaemic activity of the plant extract.

The plant extract of *Barleria longiflora* didn't show any mortality and toxicity even at highest dose of 200mg/kg body weight employed. Hence, present research study was carried out using dose 400mg/kg body weight.

OGTT referred to as the glucose tolerance test, measures the body's ability to metabolize glucose, or clear it out of the blood stream. The test reveals how quickly glucose is metabolized from the blood stream for use by cells as an energy source. The methanol extract of the leaf and fruit part of plant of *Barleria longiflora* produced hypoglycemia and improved glucose tolerance in diabetic rat's inspite of counter regulatory factors avoiding reduction in blood glucose levels.

Therefore, hypoglycemic activity of MBG could be mediated by stimulation of surviving beta cells to release more insulin and may be through extra-pancreatic mechanisms. The MBG (400mg /kg) dose showed promising results.
Like the plant extract, Metformin also produced a significant reduction in the blood glucose level of diabetic rats. Metformin exert its action mainly by increasing the secretion of insulin. They only work in diabetics with some remaining beta cells. They bind to the ATP-inhibited K^+channels in the beta cell membranes and inhibit channel activity, depolarizing the beta cell membrane and increasing Ca^{2+} influx and hence insulin release.

CONCLUSION:

Phytochemical screening of the extract shows the presence of chemical constituents like Alkaloids, steroids, fixed oils, cardio tonic a glycones, flavonoids, saponins, carbohydrates, proteins, resins. Acute toxicity tests were performed according to the OECD guide line no.423, LD50 value was found to be 200mg/kg and 400mg/kg.

Anti Hyperlipidaemic activity was performed by using the high fat diet and Triton-x-100 induced method. In the present study an increase in plasma HDL-cholesterol with a concomitant percentage decrease from other 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 Barleria longiflora And total proteins which is actually lowered in Triton-x-100 can be raised significantly with *Barleria longiflora***.** Atherogenic index which actually raised in atherogenic diet and Triton-x-100, can be lowered significantly with Barleria longiflora and a very good % protection was seen with *Barleria longiflora* 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 *Barleria longiflora* possesses significant hypoglycaemic activity and anti Hyperlipidaemic activity, which is the first claim in this respect.

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