Effect Of Ethanolic Extract Of *Cardia Dichotma* On Streptozotocin Induced Diabetic Cardiomyopathy In Rats

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Abstract:

Diabetes is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger)· Diabetes mellitus is a syndrome characterized by hyper-glycaemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion and, or insulin action. About 70-80% of deaths in diabetic patients are due to vascular disease. In particular hyperglycemia, the primary clinical manifestation of diabetes, is thought to contribute to diabetic complications by altering vascular cellular metabolism, vascular matrix molecules and circulating lipoproteins. STZ is a nitrosourea analogue, preferentially uptake by pancreatic beta cells via GLUT2 glucose transporter and causes DNA alkylation followed by the activation of poly ADP ribosylation leading to depletion of cytosolic concentration of NAD^+ and ATP. The present study was performed to evaluate the potency and effect of ethanolic extract of *Cardia dichotma* in diabetic Cardiomyopathy. Thus, it can be concluded from our findings that the levels of Glucose, total, total serum cholesterol, triglycerides, total lipids, VLDL and LDL-cholesterol which are actually raised in diabetic cardiomyopathy can be lowered with ethanolic extract of *Cardia dichotma*. Level of body wt; total protein, serum albumin, serum globulin, and HDL decreased in diabetes cardiomyopathy can be increased with ethanolic extract of *Cardia dichotma.* Two doses were given to chosen animal model and dose at 200mg/kg has shown its significant effect. Further studies both on the extract and/or its chemical constituents are needed to pinpoint the findings.

Keywords: - Diabetic Cardiomyopathy, *Cardia dichotma,* DNA alkylation, GLUT2 glucose transporter.

INTRODUCTION

Diabetes

Diabetes is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger) .

Types of Diabetes

Diabetes mellitus is a syndrome characterized by hyper-glycaemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion and, or insulin action.

Type 1

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of langerhans in the pancreas, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which beta cell loss is a T-cell- mediated autoimmune attack. There is no known preventive measure against type 1 diabetes, which causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed "juvenile diabetes" because a majority of these diabetes cases were in children.

Type 2

Type 2 diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. Type 2 diabetes is the most common type. In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver.

Diabetes insipidus is a condition characterized by excessive thirst and excretion of large amounts of severely diluted urine, with reduction of fluid intake having no effect on the concentration of the urine. There are different types of DI, each with a different set of causes. The most common type in humans is the neurological form, called Central DI (CDI), which involves a deficiency of arginine vasopressin (AVP), also known as antidiuretic hormone (ADH). The second common type of DI is nephrogenic diabetes insipidus (NDI), which is due to kidney or nephron dysfunction caused by an insensitivity of the kidneys or nephrons to ADH. The incidence of diabetes insipidus in the general population is three in 100,000.

Sign and Symptoms: -Symptoms may include Polyuria, Polydipsia, Polyphagia, Weight loss, Fatigue, Cramps, Constipation, Blurred vision, Candidiasis etc.

Cardiomyopathy:- Cardiomyopathy (literally "heart muscle disease") is the measurable deterioration for any reason of the ability of the myocardium (the heart muscle) to contract, usually leading to heart failure. Common symptoms include dyspnea(breathlessness) and peripheral edema (swelling of the legs). Those with cardiomyopathy are often at risk of dangerous forms of irregular heart rate and sudden cardiac death. The most common form of cardiomyopathy is dilated cardiomyopathy. Although the term "cardiomyopathy" could theoretically apply to almost any disease affecting the heart, it is usually reserved for "severe myocardial disease leading to heart failure".

Cardiomyopathies are either confined to the heart or are part of a generalized disorder, both often leading to death or progressive heart failure. Other diseases that Cause heart muscle dysfunction are excluded, such as coronary artery disease, hypertension, or abnormalities of the heart valves.

Symptoms may include shortness of breath after physical exertion, fatigue, and swelling of the feet, legs, or abdomen. Additionally, arrhythmias and chest pain may be present. The pathophysiology of cardiomyopathies is better understood at the cellular level with advances in molecular techniques. Mutant proteins can disturb cardiac function in the contractile apparatus (or mechanosensitive complexes). Cardiomyocyte alterations and their persistent responses at the cellular level cause changes that are correlated with sudden cardiac death and other cardiac problems.

Plant Profile

Cordia dichotoma, commonly known as the Indian cherry or glue berry tree, is a small to moderate-sized deciduous tree in the borage family (Boraginaceae). It is native to the Indomalayan realm, northern Australia, and western Melanesia. The tree is well-known for its edible fruit and extensive use in traditional medicine systems like Ayurveda, Unani, and Siddha.

Medicinal Uses: The plant is a rich source of phytochemicals such as flavonoids, tannins, alkaloids, and phenolic compounds, which contribute to its medicinal properties. It has been traditionally used to treat a wide range of ailments, including coughs, chest complaints, stomach aches, and inflammatory conditions.

RESEARCH ENVISAGED

Diabetic cardiomyopathy is the secondary complication of Diabetic mellitus and Diabetes insipidus. Diabetic cardiomyopathy is a disorder that affects the heart rate and blood pressure due to structural abnormality in cardiovascular system.

Cardia dichotoma is known to be having potent anti-diabetic and anti- hyperlipedimic activity. These properties of *Cardia dichotoma* makes it beneficial in improving cardiac profile in Diabetic cardiomyopathy. Effect of *Cardia dichotoma* on Cardiomyopathy as a secondary complication of Diabetes has not been yet studied. So we are trying to explore the effect and potency of Ethanolic extract of *Cardia dichotoma*.

MATERIAL AND METHOD

S.No.	Chemicals	Drugs	Instruments
1.	**Materials** Petroleum ether Ethanol **Diagnostic kits** Biuret kit for protein estimation	Streptozotocin *Cardia dichotma* extract Metformine B-Blocker (Metolol)	Glucometer Soxhler apparatus Bioanalyser Calorimeter
2.	Span diagnostic kit		

Pharmacognostic study: -

Collection & authentication: - The leaves of *Cardia dichotma* has been taken from local market, authenticated from department of botany Dr. H.S. Gour University, Sagar (M.P.). Herbarium no. Bot./Her. /2023 and reference no. 32/Bot./168.

Extraction Method

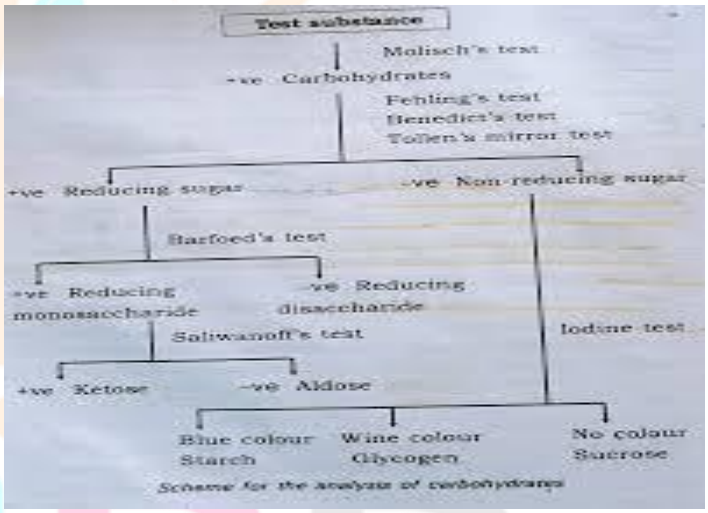
Petroleum ether: The shad dried coarse powder of bark were packed in extraction thimble of soxhlet apparatus and subjected to continuous hot extraction with petroleum ether for 18 hour or till the clear extraction was obtained. The extract was filtered while hot and resultant extract was distilled in vacuumed under reduced pressure in order to remove the solvent completely. It was dried and kept in the desiccator till the experimentation. Obtained extract was weighed and percentage yield were calculated in terms of air-dried powdered crude material.

Ethanol extract: Mark left after petroleum ether dried below 55 ^{0}C (hot air oven) and then packed well in extraction thimble of soxhlet apparatus and subjected to continuous hot extraction with petroleum ether for 18 hour or till the clear extraction was obtained. The extract was filtered while hot and resultant extract was distilled in vacuumed under reduced pressure in order to remove the solvent completely. It was dried and kept in the dessicator till the experimentation. Obtained extract was weighed and percentage yield were calculated in terms of air-dried powdered crude material.

Phytochemical Screening: Test for Alkaloid

Test	Reagent Composition	Positive Colour Change
Dragendorffs Reagent	Potassium bismuth iodide	Reddish-brown
Mayer's Reagent	Potassio mercuric iodide	White or pale yellow ppt
Hager's Reagent	Picric acid	Yellow
Wagner's Reagent	Solution of iodine in potassium iodide	Yellow or brown ppt.
Murexide Test (for Caffeine and Other Purine Derived Alkaloids)	Potassium chlorate + drops of HCl. Expose the resultant to NH_3	Purple colouration

Test for Carbohydrates



Tests for Glycosides:

	Test	Observation	Inference
Cardiac glycosides A	LEGAL TEST 2ml of extract + 2ml of pyridine and a few drops of 2% sodium nitropruside + 20% NaOH	Brownish colour seen	Cardenolides present
B	LIEBERMAN'S BURCHARDS TEST 2ml of extract + 2ml of acetic acid + H2SO4 (conc.) carefully added and cool	Light brownish green seen	A steroidal nucleus present
C	SALKOWSKI TEST 2ml of extract was dissolved with 2ml of chloroform + conc. H2SO4 carefully added	Deep reddish brown colour, at the interface a steroid ring seen	A glycone portion of the cardiac glycosides present.
D	KEDDE TEST 2ml of extract + 3.5 dinitrobenzoic acid in methanol + NaOH	Reddish brown ring	Lactone ring in cardenolides present
E	KELLER-KILIANI TEST 2ml of extract + 2ml of glacial acetic acid + FeCl3 + H2SO4 conc.	Greenish brown ring	A de-oxy sugar character of cardenolids present

Pharmacological Screening

Animal Model: STZ Induce Diabetes Type 2

Mature Sprague-Dawley rats (200–225gm) were taken from the animal house of SIPS, Sagar. All animal were kept in standard plastic polypropylene cages with stainless steel coverlids and wheat straw was used as bedding material. The animal were facilitated with standard environment of photoperiod (12:12 hr dark: light cycle) and room temperature (23 ± 2^0 C). The animal assists free to feed and purified water ad libitum. All experiment was according to CPCSEA (SIPS/EC/2023/59) guidelines. Animals fast for overnight and streptozotocin (STZ; 65 mg/kg) in 0.02 M citrate saline buffer were administered intraperitoneally. The age-matched control group received citrate buffer only. Blood glucose levels were monitored every week. Rats with blood glucose levels ≥15 mM (200 mg/dl) for 2 consecutive weeks was considered diabetic.

Description of groups Control Group (Vehicle Treated) **Negative Control** (Disease Induced) **Standard** (Metformin)+ Metolol

Test group-1 (Ethanolic Extract of *Cardia dichotma* 100mg/kg)

Test group-II (Ethanolic Extract of *Cardia dichotma* 200mg/kg)

Evaluating Parameters

Body weight Estimation

Body weight of all the animals measured every week till the completion of study by weighing balance.

Biochemical estimation

Estimation of Blood Glucose

Blood samples were collected by retro-orbital sinus/plexus bleeding method; in which, in anaesthetized animal tip of capillary tube was gently inserted below the eye at approximately a 45-degree angle into the space between the globe and the lower eyelid. At a point; capillary tube feels rest on the orbit, at that time tube was twisted between thumb and forefinger. After that, sinus/plexus will rupture and blood was flow through the tube, blood sample was collected and multiple tubes required collecting total volume. Blood glucose level was measure by gluco-meter. The blood glucose estimation was done weekly after administration of test compound.

Estimation of Serum protein

Total Serum Protein Estimation:- a). 5ml of Biuret reagent were pipette out into each test tube.

Pipette 5ml of the Biuret blank reagent were pipette out into each test tubes.

Reagent-series were prepared by adding 100 µl of each of the protein standards to five separate test tubes fill with the Biuret reagent. Reagent blank was prepared by adding 100 µl of water to a sixth different test tube filled with Biuret reagent. Serum unknown was prepared by adding 100 µl of serum to a seventh test tube fill with Biuret reagent. Each tube mixed by placing a piece of a para-film on the top and inverting several times.

Blank-series was prepared by adding 100 µl of each of the protein standards to five separate test tubes fill with the Biuret blank reagent. Reagent blank were prepared by adding 100 µ of water to a sixth different test tube filled with Biuret blank reagent. Serum unknown was prepared by adding 100 µl of serum to a seventh test tube fill with Biuret blank reagent. Each tube was mixed by placing a piece of a para-film on the top and inverting several times.

Cuvettes were allowed to stand at room temperature for 30 minutes.

Reagent-series blank was use, zero the Spec 20 at 540 nm and absorbance of the reagent series will measure including the serum unknown. All the test tube was inverted before that measurement.

Blank-series blank were use, re-zero the Spec 20 and absorbance of the blank series including the serum unknown was measured.

Blank subtraction were conduct by subtracting the absorbance of the blank-series from its reagent series counterpart. Concentration of the unknown was determined by the plotting of graph between absorbance vs. concentrations.

Albumin: 1. With an Ostwald pipette calibrated to "blow out", measure 0-5 ml. serum into a test-tube and add 9.5 ml. of 27.79 % ammonium sulphate (the resulting mixture is 23M).

Mix, filter through No. 44 Whatman filter-paper, and when the filtrate comes through clear refilter through the same paper.

Measure 5 ml of the filtrate into a centrifuge-tube and add 5 ml 10 % trichloroacetic acid, mix, and centrifuge for 3 minutes at about 3000 revolutions per minute.

Decant the clear supernatant fluid: add 1 ml. 30 % NaOH and about 3 ml. distilled water and shake: when the protein has dissolved add more water up to 9 ml. then 5 % $CuSO_4$ up to 10 ml.

Shake for 10 seconds. Centrifuge as before. Decant the supernatant fluid for comparison in the colorimeter with the standard.

Calculation: If the unknown is set at 20 mm. and the readings of the standard for total protein and albumin are X and Y mm. respectively, then

Total protein= 0.3X % Albumin = 0.24Y %

Globulin = (0.3X- 0.24 Y) %.

Estimation of Serum lipid profile:

- **Total cholesterol:** For estimation of total-cholesterol a kit containing: -

1) Cholesterol reagent and 2) Cholesterol standard will be used, reaction followed CHOD-PAP method.

Procedure: Three different following solutions will be taken they are:

Blank: contained 1000 µl of reagent 1.

Standard: contained 1000 µl of reagent 1 and 10 µl of reagent 2.

Test: contained 1000 µl of reagent 1and 10 µl of test serum. Mixed well, incubate at 37ºC for 10 minute or at room temperature (15-30ºC) for 30 minutes. Programme the analysers as per assay parameters given in kit.

Blank the analysers with reagent blank.

Measure absorbance of standard followed by the test at wavelength 505 nm.

Calculated the results as per given calculation formula.

Calculation:

Absorbance of Test Cholesterol concentration (mg/dl) =× 200

Absorbance of standard`

Triglyceride (TG)

Triglyceride were estimate using accurate Triglycerides kit of Span Diagnostics Pvt. Ltd. Accurately triglycerides estimation kit is formulated using GPO (Glycerol-3 Phosphate Oxidase) and peroxide for quantitative estimation of serum triglycerides. The kit contains 2 reagents i.e.1) Triglycerides mono reagent and 2) Triglycerides standard.

Preparation of working Reagent: The contents of enzyme reagent will be dissolved in 10 ml of diluents buffer. The working reagent is stable for 4 - 6 week at 2 - 8ºC.

Procedure: 3 different following solutions will be taken they are:

Blank: contained 1000 µl of reagent 1.

Standard: contained 1000 µl of reagent 1 and 10 µl of reagent 2.

Test: contained 1000 µl of reagent 1and 10 µl of test serum Mixed and incubated at 37ºC for 10 minutes. Programmed the analyzers as per assay parameters given in kit.

Blank the analyzer with Reagent Blank.

Measure absorbance of standard followed by the test.

Calculate results as per given calculation formula.

Absorbance of Test

Calculation: Triglycerides (mg/dl) =× 200

Absorbance of Standard

High-density lipoprotein (HDL)

For estimation of High-density lipoprotein, a kit containing:

Cholesterol reagent

Precipitating reagent PEG-6000

HDL-cholesterol standard were used, reaction followed PEG-CHOD-PAP method, end point assayed with Lipid clearing factor (LCF).

Preparation of working reagents: Dissolve enzyme reagent with 25 ml. of diluents buffer and kept for at least 10 min. before use. The working reagent is stable for 4 weeks at 2-8ºC.

Procedure: 200µl of clear serum will be poured into tubes added 200 µl of reagent to mix well and incubated at temperature for (15-30ºC) for 10 min for HDL-cholesterol separation. Kept all tubes in cooling centrifugation chamber at 2000 rpm for 15 minute and separate clear supernatant which was used for HDL-Cholesterol estimation. For HDL-Cholesterol estimation. Different following solutions have been prepared:

Blank: contained 1000 µl of reagent 1.

Standard: contained 1000 µl of reagent 1and 100 µl of reagent 3. Test: contained 1000 µl of reagent 1 and 100 µl of test serum. Mixed well, incubate at 37ºC for 10 minute or at room temperature (15-30ºC) for 30 minutes. Programme the analyzers as per assay parameters given in kit.

Blank the analysers with reagent blank.

Measure absorbance of standard followed by the test at wavelength 505 nm.

Calculated the results as per given calculation formula.

Calculation

- Absorbance of Test
- Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL
- Cardiac Marker

Histopathology

The heart tissue obtained from different group were immediately washed with saline and then fixed in 10% v/v formalin. The ventricular mass from left ventricle was sectioned in order to obtain 0.4 cm thick transverse section and dehydrated with alcohol followed by embedding in paraffin wax. These sections was stained with heamatoxylin and eosine (H and E).

OBSERVATION, RESULT & DISCUSSION

Pharmacological Screening

Body Weight

Group	Average body weight (gm)					
	0 day	7th day	14th day	21st day	28th day	35th day
V.C.	155±3.5610	160±3.2218	157±4.3813	165±2.5610	165±2.3814	167±2.5716
N.C.	160±3.5125	150±3.2244	135±3.3125	128±3.6157	118±3.5234	105± 3.7388
Std	155±3.4714	150±3.2186	133±3.9358	125±3.4235***	122±3.4156***	122±3.3344***
T1	155±3.4305	147±3.5125	135±3.7246	130±3.2248**	128±3.3316***	128±3.4086***
T2	163±3.4513	150±3.2208	132±3.7088	127±3.3082***	125±3.4238***	125±3.2056***

Table No. 1. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- *Cardia dichotma* (100mg/kg), T2- *Cardia dichotma* (200mg/kg).Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, a*** = P<0.001 when compared to negative control group.

Discussion

Diabetic cardiomyopathy results in loss of body weight. Decrease in the body weight due to derangement of metabolic pathways is a common feature in diabetes. The body weight of control rats was progressively increased (155±3.5610 gm to 167±2.5716 gm) whereas there was a significant decrease in the body weight (160±3.5125 gm to 105±3.7388 gm) of STZ induced diabetic rats, which might be due to the breakdown of tissue proteins. Diabetic rats treated with dose of *Cardia dichotma* from 14th day at 100mg/kg and 200mg/kg shows no weight gain, indicating the beneficial effect of the *Cardia dichotma*.

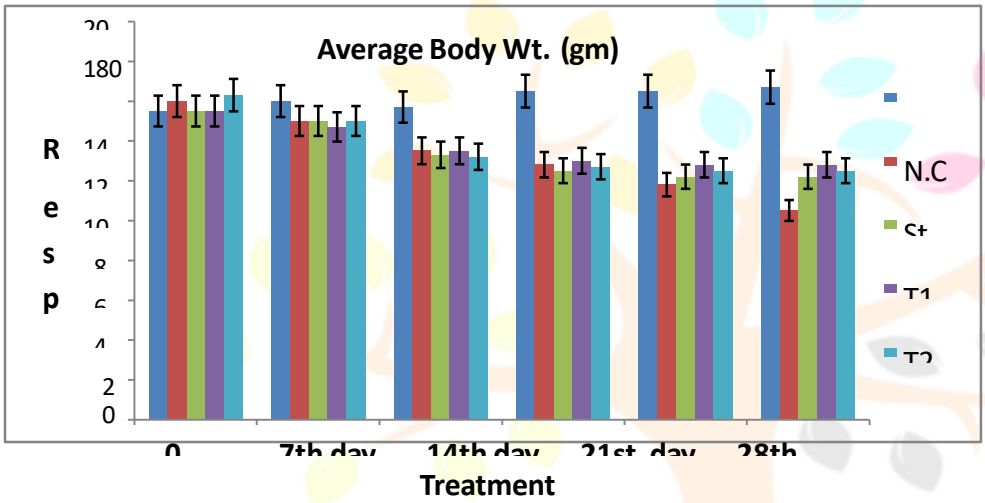


Fig 1. Graphical representation of Body weight. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- Test group I (*Cardia dichotma* 100mg/kg), T2- Test group II (*Cardia dichotma* 200mg/kg).

2 Blood Glucose Level

Table No. 2. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- *Cardia dichotma* (100mg/kg), T2- *Cardia dichotma* (200mg/kg). Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, a*** = P<0.001 when compared to negative control group.

Group	Blood Glucose Level (mg/dl)					
	0 day	7th day	14th day	21st day	28th day	35th day
V.C.	85±2.4125	85±3.1215	85±2.3218	83±4.1230	85±2.3216	85±2.3269
N.C.	86±2.4256	164± 3.4245	218±2.7216	225±3.9254	238±2.7256	257±2.4503
Std	85±2.5233	163± 3.5205	217±2.7213	197±3.8246**	162±2.6804***	158±2.5304**
T1	85±2.4245	167± 3.6202	215±2.6214	205±3.7286**	185±2.3505***	178±2.3604**
T2	86±2.3218	165± 3.6254	218±3.4288	195±4.2246**	175±2.5303***	162±2.3704**

Discussion

Blood glucose level is an index for the diagnosis of diabetic cardiomyopathy. Hyperglycaemia is an important factor in the development and progression of the complications of diabetic cardiomyopathy**.** In the present study, diabetic rats showed elevation in blood glucose level (86±2.4256mg/dl to 257±2.4503mg/dl) which upon oral administration of ethanolic extract of *Cardia dichotma* resulted in a significant reduction of blood glucose level (P>0.001) dose of 100mg/kg (215±2.6214 mg/dl to 178±2.3604 mg/dl) and 200mg/kg (218±3.4288 mg/dl to 162±2.3704 mg/dl), indicating the beneficial effect of the *Cardia dichotma*.

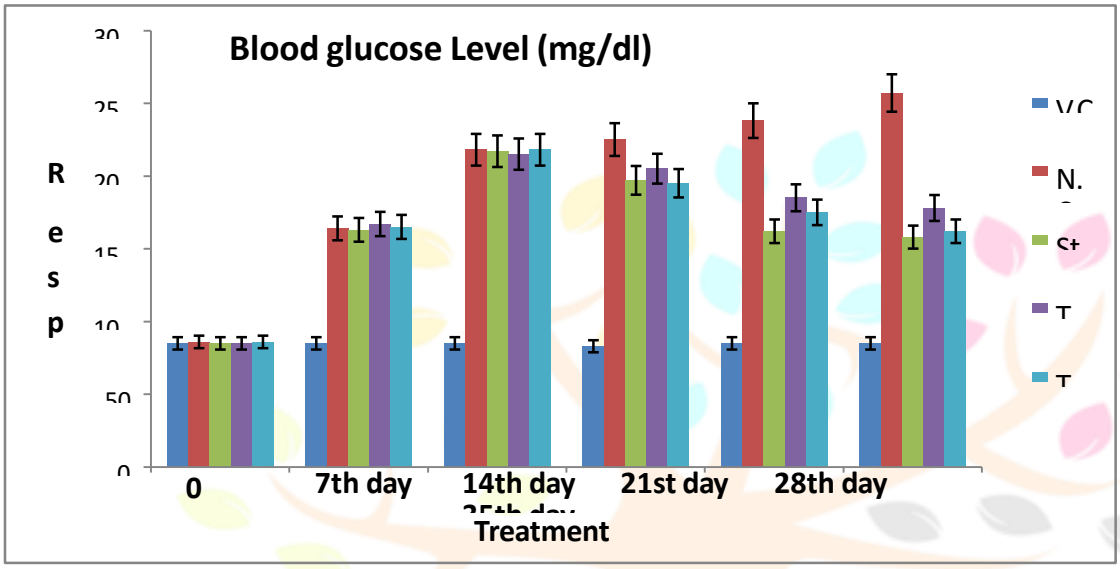


Fig 2. Graphical representation of Blood Glucose Level. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- Test group I (*Cardia dichotma* 100mg/kg), T2- Test group II (*Cardia dichotma* 200mg/kg).

Total Serum Protein

Table No. 3. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- *Cardia dichotma* (100mg/kg), T2- *Cardia dichotma* (200mg/kg).Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, a*** = P<0.001 when compared to negative control group.

Group	Total protein in serum (gm/dl)					
	0 day	7th day	14th day	21st day	28th day	35th day
V.C.	6.27± 0.3121	6.28± 0.1245	6.22±/0.3215	6.21±0.3616	6.21±0.3251	6.22±0.3254
N.C.	6.25± 0.4153	5.65± 0.2153	2.72±0.2258	2.27±0.3351	2.34±0.3216	1.94±0.3242
Std	6.26± 0.3162	5.64± 0.2542	2.68±0.2263	3.71±0.3252***	3.98±0.3276**	5.65±0.3245***
T1	6.26± 0.3165	5.62± 0.1328	2.65±0.2256	3.63±0.3255***	3.68±0.3258**	4.85±0.3267***
T2	6.22± 0.3546	5.58± 0.2351	2.62±0.2254	3.72±0.3248***	3.87±0.4052**	5.18±0.3058***

Discussion

The level of total proteins is found to decrease in diabetic group of rats (6.25±0.4153gm/dl to 1.94±0.3242gm/dl). The deficiency of insulin leads to defective amino acid/protein metabolism, which may be a more important factor than hyperglycaemia in the etiology of some diabetic complications. During our present study, upon oral administration with *Cardia dichotma* resulted in a significantly inhibits proteolysis caused by insulin deficiency and thus increased the levels of total proteins at the dose of dose of 100mg/kg (2.65±0.2256gm/dl to 4.85±0.3267gm/dl) and 200mg/kg (2.62±0.3248gm/dl to 5.18± 0.3058gm/dl), indicating the beneficial effect of the *Cardia dichotma*.

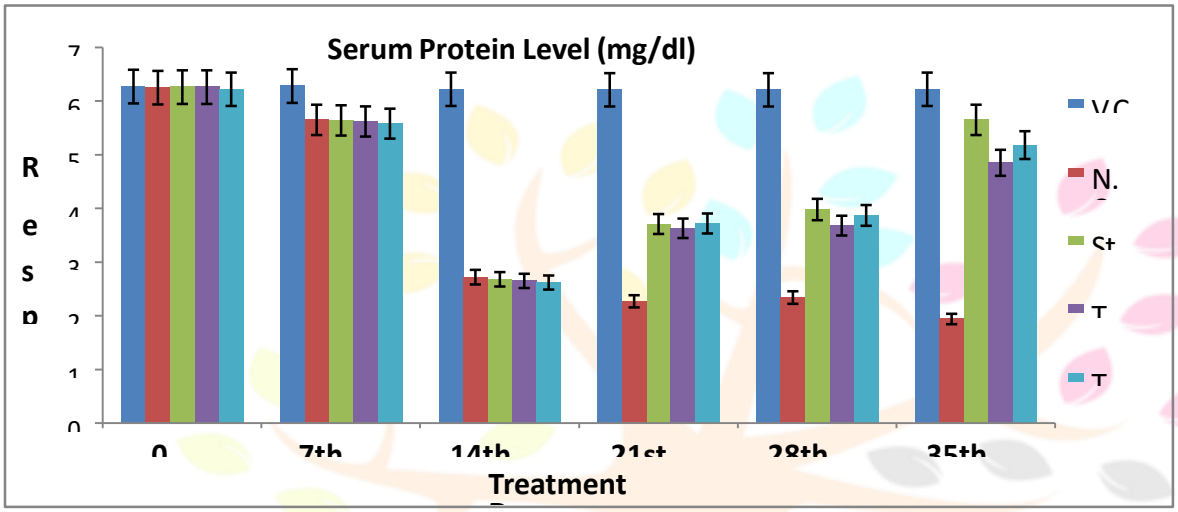


Fig3. Graphical representation of Total Serum Protein Level. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- Test group I (*Cardia dichotma* 100mg/kg), T2- Test group II (*Cardia dichotma* 200mg/kg).

Serum Albumin Level

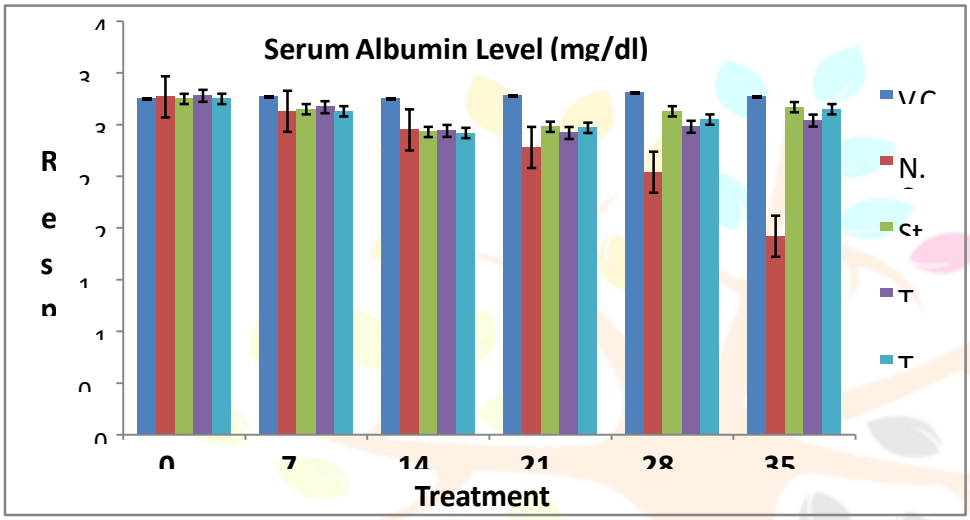
Table no. 4. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- *Cardia dichotma* (100mg/kg), T2- *Cardia dichotma* (200mg/kg). Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, a*** = P<0.001 when compared to negative control group.

Group	Serum Albumin Level (gm/dl)					
	0 day	7th day	14th day	21st day	28th day	35th day
V.C.	3.25±0.18	3.27±0.23	3.25±0.15	3.28±0.27	3.31±0.23	3.27±0.26
N.C.	3.27±0.18	3.13±0.23	2.95±0.17	2.78±0.26	2.54±0.21	1.92±0.23
Std	3.25±0.21	3.15±0.18	2.93±0.23	2.98±0.25***	3.13±0.18***	3.17±0.22***
T1	3.28±0.23	3.17±0.21	2.94±0.17	2.92±0.23***	2.98±0.25***	3.04±0.26***
T2	3.25±0.28	3.13±0.23	2.92±0.26	2.97±0.18***	3.05±0.27***	3.15±0.25***

Discussion

The level of Albumin which is responsible for maintaining osmotic pressure was found to decrease in diabetic group of rats (3.27±0.18gm/dl to 1.92±0.23gm/dl). Insulin deficiency leads to defective metabolism of fatty acids, proteins and carbohydrates**.** Our Study says that upon oral administration of *Cardia dichotma* resulted in a significantly inhibition of proteolysis caused by insulin deficiency and thus increased the levels of albumin at the dose of dose of 100mg/kg (2.94±0.17gm/dl to 3.04±0.26gm/dl) and 200mg/kg (2.92±0.26gm/dl to 3.15±0.25gm/dl), indicating the beneficial effect of the *Cardia dichotma*.

Fig 4. Graphical representation of Serum Albumin Level. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- Test group I (*Cardia dichotma* 100mg/kg), T2- Test group II (*Cardia dichotma* 200mg/kg).



Serum Globulin Level

Table No. 5. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- *Cardia dichotma* (100mg/kg), T2- *Cardia dichotma* (200mg/kg).Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, a*** = P<0.001 when compared to negative control group.

Group	Serum Globulin Level (gm/dl)					
	0 day	7th day	14th day	21th day	28th day	35th day
V.C.	2.12±0.13	2.11±0.08	2.07±0.17	2.06±0.09	2±0.08	2.05±0.08
N.C.	2.08±0.25	1.62±0.00	0.87±0.11	0.49±0.05	0.35±0.15	0.42±0.09
Std	2.11±0.11	1.59±0.04	0.85±0.00	0.93±0.07***	0.95±0.18***	1.78±0.13***
T1	2.10±0.12	1.55±0.03	0.81±0.09	0.71± 0.12***	0.80±0.13***	1.21±0.11***
T2	2.07±0.08	1.55±0.02	0.80±0.02	0.78± 0.2***	0.79±0.15***	1.43±0.13***

Discussion

The globulins serve as antibodies and transport substances. It involves in defense mechanism. The level of globulin was found to decrease in diabetic group of rats (2.08±0.25gm/dl to 0.42±0.09gm/dl). Insulin deficiency leads to defective metabolism of fatty acids, proteins and carbohydrates**.** Our Study says that upon oral administration of *Cardia dichotma* resulted in a significantly inhibition of proteolysis caused by insulin deficiency and thus increased the levels of globulin at the dose of dose of 100mg/kg (0.81±0.09gm/dl to 1.21±0.11gm/dl) and 200mg/kg (0.80±0.02gm/dl to 1.43±0.13gm/dl), indicating the beneficial effect of the *Cardia dichotma*.

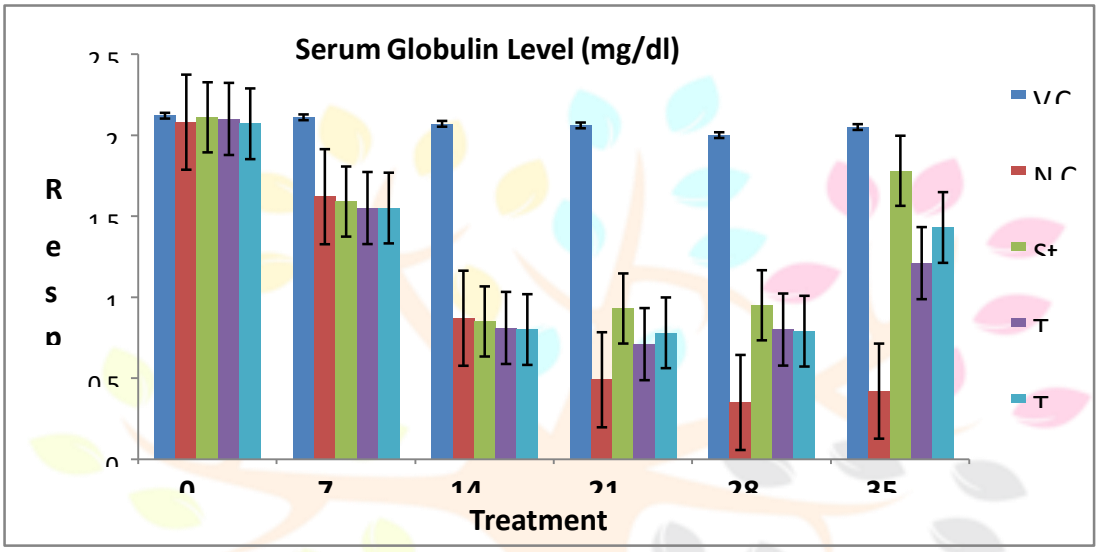


Fig 5. Graphical representation of Serum Globulin Level. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- Test group I (*Cardia dichotma* 100mg/kg), T2- Test group II (*Cardia dichotma* 200mg/kg).

Serum HDL Level

Table No. 6. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- *Cardia dichotma* (100mg/kg), T2- *Cardia dichotma* (200mg/kg).Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, a*** = P<0.001 when compared to negative control group.

Group	Serum HDL Level (mg/dl)					
	0 day	7th day	14th day	21st day	28th day	35th day
V.C.	53.6±2.64	53.4±2.71	54.1±2.73	53.5±2.91	53.2±2.70	53.5±2.72
N.C.	54.7±2.67	48.4±2.64	46.2±2.52	43.6±2.69	41.2±2.71	34.6±2.58
Std	54.1±2.79	46.1±2.65	45.4±2.64	45.1±2.60***	49.6±2.71***	51.3±2.64***
T1	53.2±2.70	46.9±2.52	43.8±2.67	44.7±2.63***	46.8±2.73***	47.2±2.53***
T2	54.3±2.68	45.8±2.37	41.5±2.36	45.3±2.59***	48.2±2.59***	49.1±2.92***

Discussion

Alterations in serum lipid profiles are known in diabetics, which are likely to increase the risk of heart disease. Diabetic Cardiomyopathy is associated with elevated levels of TC, TG, LDL, VLDL and a fall in the level of HDL. This abnormal state can be reversed to normal on administration of Ethanolic extract of *Cardia dichotma*. HDL level decreases (54.7±2.67mg/dl to 34.6±2.58mg/dl) in negative control group, which upon oral administration of ethanolic extract of *Cardia dichotma* at dose of 100mg/kg (43.8±2.67mg/dl to 47.2±2.53mg/dl) and 200mg/kg (41.5±2.36mg/dl to 49.1±2.92mg/dl) resulted in a significant elevation (P>0.001) of HDL level.

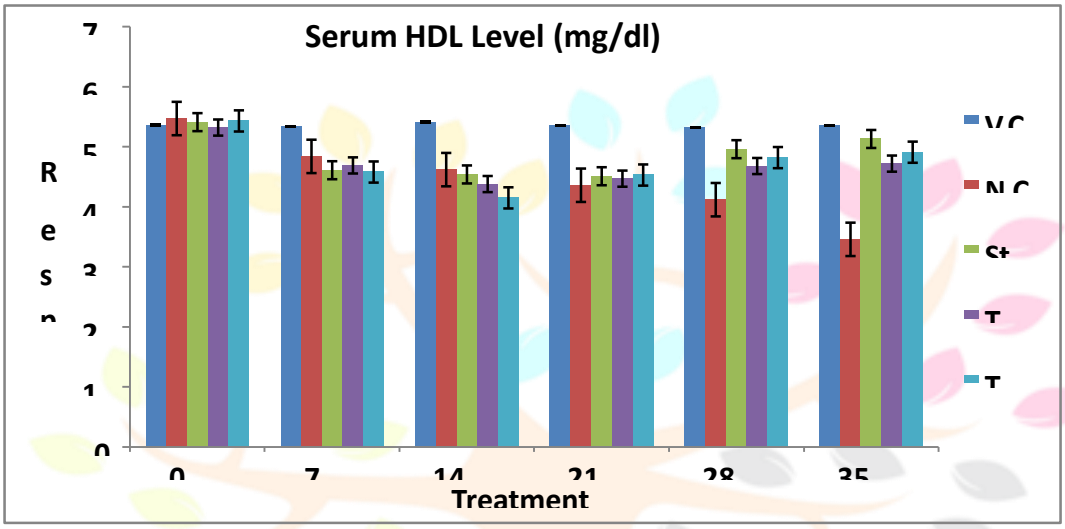


Fig 6. Graphical representation of Serum HDL. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- Test group I (*Cardia dichotma* 100mg/kg), T2- Test group II (*Cardia dichotma* 200mg/kg).

Serum LDL Level

Table No. 7. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- *Cardia dichotma* (100mg/kg), T2- *Cardia dichotma* (200mg/kg).Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, a*** = P<0.001 when compared to negative control group.

Group	Serum LDL Level (mg/dl)					
	0 day	7th day	14th day	21st day	28th day	35th day
V.C.	26.4±2.31	25.3±2.37	25.6±2.45	26.8±2.42	26.7±2.43	25.4±2.46
N.C.	26.4±2.37	33.4±2.44	37.4±2.54	39.5±2.53	44.2±2.48	49.7±2.61
Std	26.2±2.42	32.7±2.43	35.8±2.53	33.2±2.32***	32.8±2.69***	26.6±2.31***
T1	26.8±2.55	31.4±2.54	34.7±2.63	33.5±2.36***	31.8±2.38***	28.4±2.91***
T2	26.3±2.53	31.8±2.55	33.9±2.65	32.6±2.61***	30.9±2.53***	27.7±2.49***

Discussion

LDL level is also elevated (26.4±2.37mg/dl to 49.7±2.61mg/dl) in negative control group, which upon oral administration of ethanolic extract of *Cardia dichotma* resulted in a significant reduction of LDL level (P>0.001) at dose of 100mg/kg (34.7±2.63mg/dl to 28.4±2.91mg/dl) and 200mg/kg (33.9±2.65mg/dl to 27.7±2.49mg/dl).

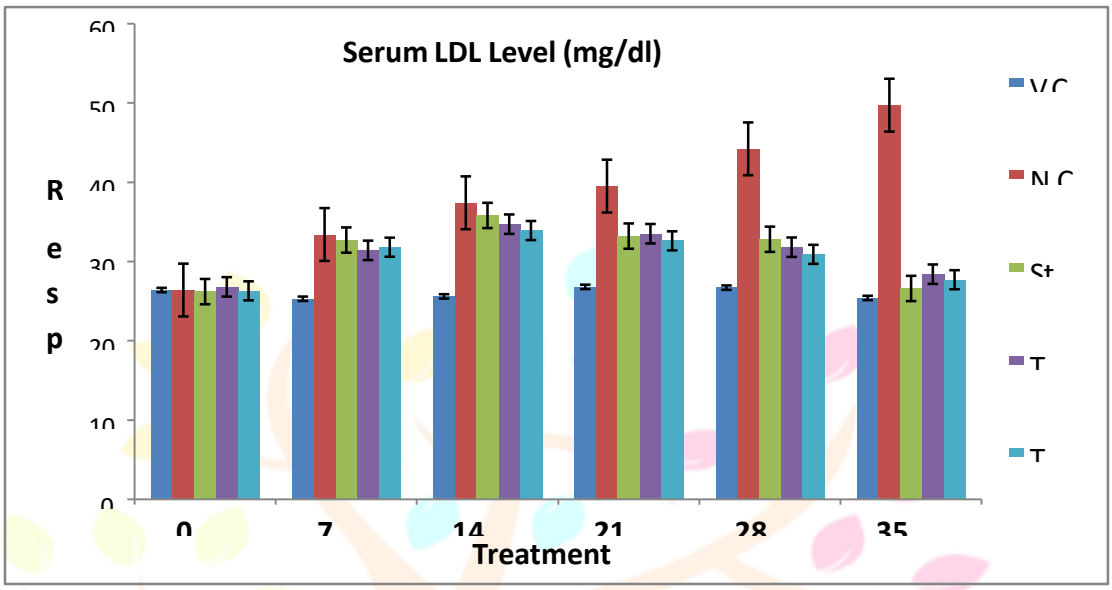


Fig 7. Graphical representation of Serum LDL Level. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- Test group I (*Cardia dichotma* 100mg/kg), T2- Test group II (*Cardia dichotma* 200mg/kg).

Serum VLDL Level

Table No. 8 V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- *Cardia dichotma* (100mg/kg), T2- *Cardia dichotma* (200mg/kg).Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, a*** = P<0.001 when compared to negative control group.

Group	Serum VLDL Level (mg/dl)					
	0 day	7th day	14th day	21st day	28th day	35th day
V.C.	23.25±2.17	23.63±2.19	23.52±2.18	23.32±2.17	23.59±2.18	23.35±2.17
N.C.	23.29±2.17	28.33±2.32	30.47±2.34	37.59±2.52	39.60±2.67	44.69±2.75
Std	23.33±2.21	27.74±2.25	29.67±2.32	26.55±2.54***	25.53±2.69***	20.89±2.36***
T1	23.55±2.18	27.96±2.21	31.87±2.54	29.25±2.33***	28.42±2.51***	25.37±2.66***
T2	23.57±2.18	28.39±2.19	30.64±2.27	27.65±2.56***	26.32±2.54***	23.85±2.71***

Discussion

Elevation in VLDL level also seen (23.29±2.17mg/dl to 44.69±2.75mg/dl) in negative control group, which resulted in a significant reduction (P>0.001) of VLDL level upon oral administration of ethanolic extract of *Cardia dichotma* at dose of 100mg/kg (31.87±2.54mg/dl to 25.37±2.66mg/dl) and 200mg/kg (30.64±2.27mg/dl to 23.85±2.71mg/dl).

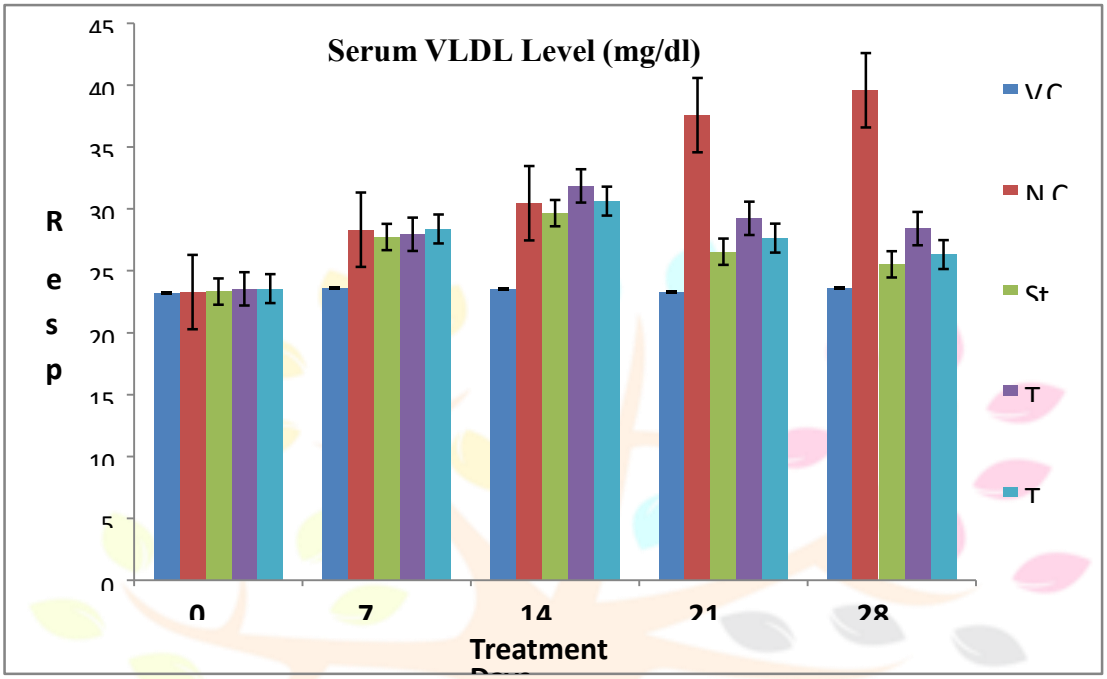


Fig 8. Graphical representation of Serum VLDL Level. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- Test group I (*Cardia dichotma* 100mg/kg), T2- Test group II (*Cardia dichotma* 200mg/kg).

Total cholesterol Level

Table No. 9. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- *Cardia dichotma* (100mg/kg), T2- *Cardia dichotma* (200mg/kg).Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, a*** = P<0.001 when compared to negative control group.

Group	Total Cholesterol Level (mg/dl)					
	0 day	7th day	14th day	21st day	28th day	35th day
V.C.	113±3.06	112±3.22	113±3.57	109±2.38	112±3.28	112±3.15
N.C.	113±3.55	125±3.24	129±3.78	132±3.54	135±3.23	142±3.17
Std	113±3.47	123±3.05	126±3.34	120±3.46***	117±3.22***	108±3.23***
T1	114±3.34	126±3.08	130±3.46	128±3.36***	127±3.26***	125±3.17***
T2	113±3.45	127±3.36	131±3.26	126±3.48***	123±3.25***	117±3.26***

Discussion

In our present study, diabetic rats showed elevation in TC level (113±3.55mg/dl to 142±3.17mg/dl) in negative control group which upon oral administration of ethanolic extract of *Cardia dichotma* resulted in a significant reduction of TC level (P>0.001) dose of 100mg/kg (130±3.46mg/dl to 125±3.17mg/dl) and 200mg/kg (131±3.26mg/dl to 117±3.26mg/dl), indicating the beneficial effect of the *Cardia dichotma*.

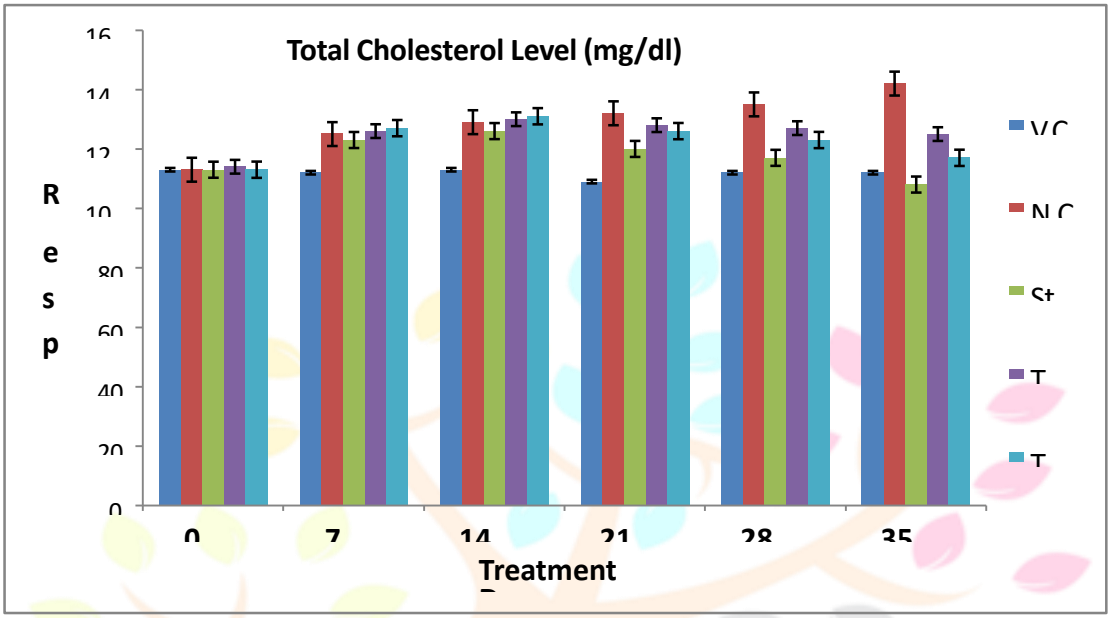


Fig 9. Graphical representation of Total cholesterol Level. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- Test group I (*Cardia dichotma* 100mg/kg), T2- Test group II (*Cardia dichotma* 200mg/kg).

Triglyceride Level

Table No. 10. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- *Cardia dichotma* (100mg/kg), T2- *Cardia dichotma* (200mg/kg).Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, a*** = P<0.001 when compared to negative control group.

Group	Triglyceride Level (mg/dl)					
	0 day	7th day	14th day	21st day	28th day	35th day
V.C	185.4±3.15	182.7±3.38	183.6±3.22	185.7±3.38	183.6±3.31	185.5±3.36
N.C.	184.8±3.45	188.9±3.76	196.6±3.56	198.6±3.54	198.9±3.43	199.6±3.21
Std	183.6±3.55	187.6±3.73	195.7±3.48	192.5±3.46***	186.3±3.32***	184.7±3.24***
T1	181.5±3.62	189.3±3.64	196.4±3.57	194.8±3.36***	193.2±3.35***	189.7±3.38***
T2	182.7±3.64	189.6±3.48	196.8±3.55	194.3±3.48***	192.3±3.36***	186.1±3.42***

Discussion

Diabetic rats showed elevation in TG level (184.8±3.45mg/dl to 199.6±3.21mg/dl) in negative control group, which upon oral administration of ethanolic extract of *Cardia dichotma* resulted in a significant reduction of TG level (P>0.001) dose of 100mg/kg (196.4±3.57mg/dl to 189.7±3.38mg/dl) and 200mg/kg (196.8±3.55mg/dl to 186.1±3.42mg/dl), indicating the beneficial effect of the *Cardia dichotma*.

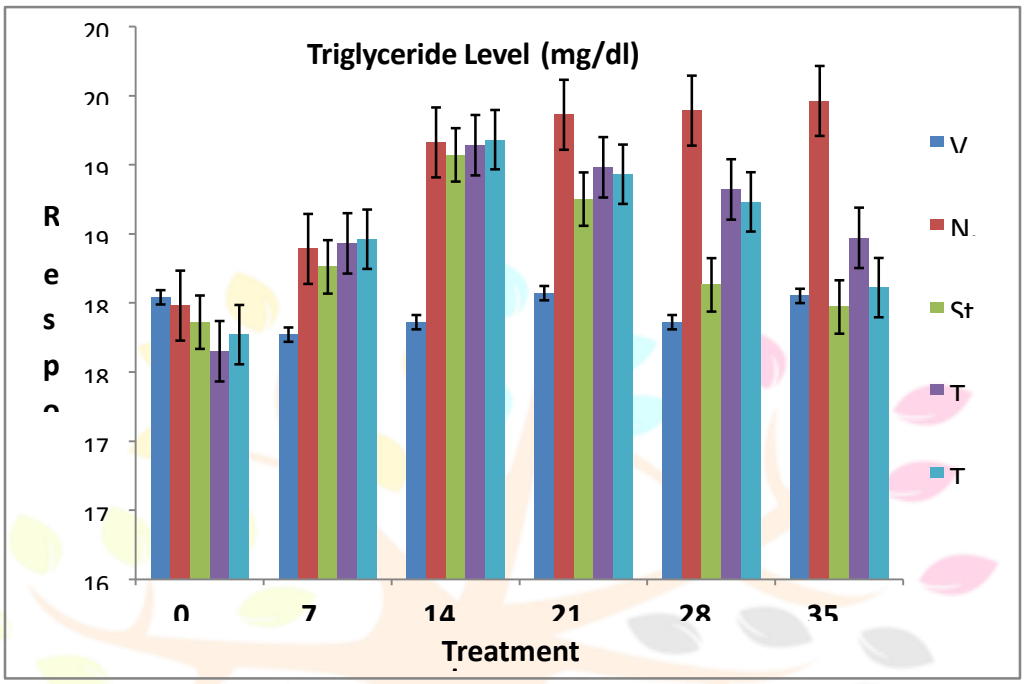


Fig 10. Graphical representation of Serum Triglyceride Level. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- Test group I (*Cardia dichotma* 100mg/kg), T2- Test group II (*Cardia dichotma* 200mg/kg).

Creatinine Kinase –MB Level

Table No. 11. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- *Cardia dichotma* (100mg/kg), T2- *Cardia dichotma* (200mg/kg).Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, a*** = P<0.001 when compared to negative control group.

Group	CK-MB Level (U/l)					
	0 day	7th day	14th day	21st day	28th day	35th day
V.C.	68.3±3.45	67.7±3.73	68.3±3.48	68.5±3.43	67.8±3.25	68.9±3.28
N.C.	67.8±3.48	85.3±3.67	92.7±33.52	93.4±3.47	97.2±3.32	98.4±3.25
Std	68.2±3.25	84.7±3.64	93.1±3.53	84.2±3.45***	78.6±3.43***	72.2±3.32***
T1	67.5±3.26	85.2±3.64	92.8±3.58	91.7±3.51***	84.5±3.48***	79.3±3.36***
T2	68.2±3.34	85.4±3.68	93.2±3.54	87.6±3.56***	75.2±3.45***	73.4±3.34***

Discussion

Elevation in CK-MB concentration in diabetic rat rise in parallel following myocardial injury and Cardiomyopathy starting to increase 4-6 h after injury, reaching peak serum concentration after 12-24 hour and baseline after 48-72 hour. Upon oral administration with *Cardia dichotma* resulted in a significantly (P>0.001) decrease the levels of CK-MB at dose of 100mg/kg (92.8±3.58U/l to 79.3±3.36U/l) and 200mg/kg (93.2±3.54U/l to 73.4±3.34U/l), indicating the beneficial effect of the *Cardia dichotma*.

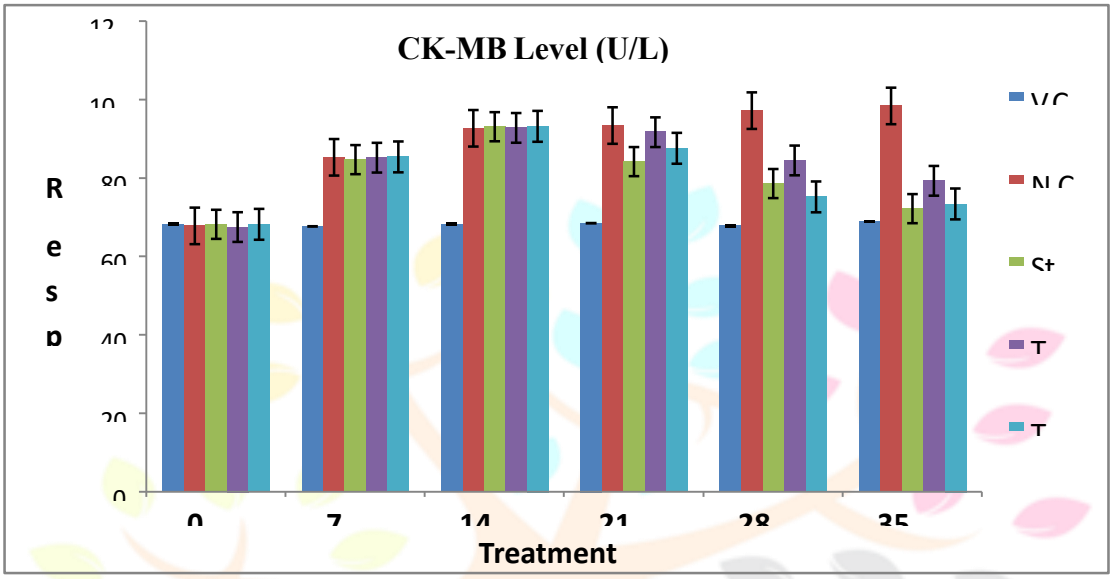
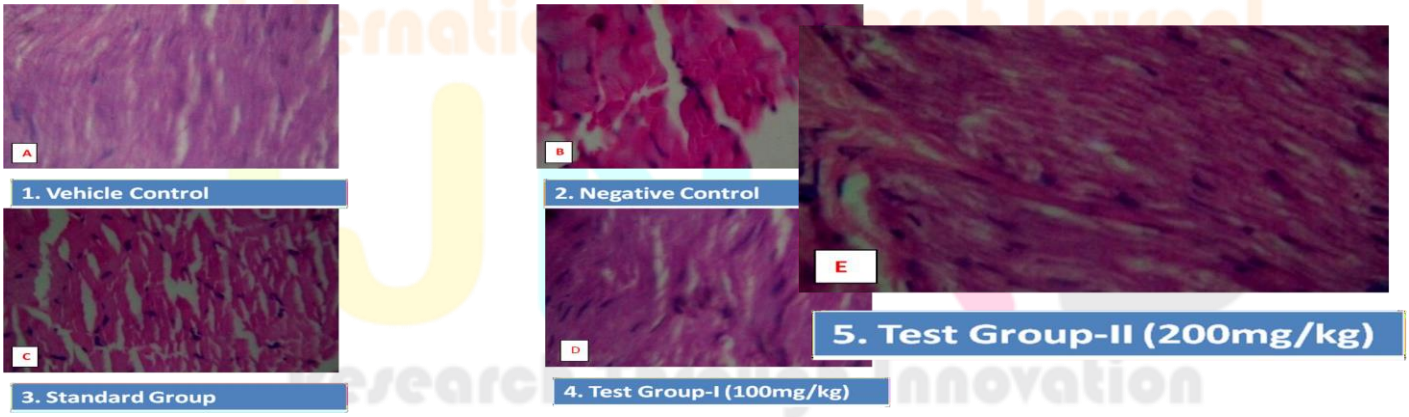


Fig 11. Graphical representation of CK-MB Level. V.C.- Vehicle Control, N.C.- Negative Control, Std- Standard, T1- Test group I (*Cardia dichotma* 100mg/kg), T2- Test group II (*Cardia dichotma* 200mg/kg).

HISTOPATHOLOGY



SUMMARY & CONCLUISION

STZ treated (65mg/kg) rats have been suggested as a novel animal model that mimics the natural history and metabolic characteristics of the common type 2 diabetes in humans, and is suitable for testing of antidiabetic compounds.

About 70-80% of deaths in diabetic patients are due to vascular disease. In particular hyperglycemia, the primary clinical manifestation of diabetes, is thought to contribute to diabetic complications by altering

vascular cellular metabolism, vascular matrix molecules and circulating lipoproteins. STZ is a nitrosourea analogue, preferentially uptake by pancreatic beta cells via GLUT2 glucose transporter and causes DNA alkylation followed by the activation of poly ADP ribosylation leading to depletion of cytosolic concentration of NAD^+ and ATP.

Diabetes is associated with quantitative changes in the amount of circulating lipids – notably an increase in triglycerides, elevated LDL and a reduction in HDL. Like other lipoproteins, HDL also undergoes significant qualitative changes in diabetes, in both structure and function. However, since dyslipidemia may be present several years before the onset of diabetes, it is hard to determine which of these changes are related to the pathognomonic features of the disease, and which precede and accelerate its progression. In fact, the role of cholesterol metabolism and HDL function in the pathogenesis of diabetic cardiomyopthy (DCM) has recently gained a lot of attention. Mice studies suggest that cholesterol accumulation in islet β-cells is the reason for their pathology. HDL also appears to protect β-cells from the toxic effects of glucose and IL- 1β, and to enhance insulin secretion. In skeletal muscles, HDL was demonstrated to increase insulin activity in the form of glucose uptake. This activity was dependent on ABCA-1 activity, and included activation of AMP kinase.

The present study was performed to evaluate the potency and effect of ethanolic extract of *Cardia dichotma* in diabetic Cardiomyopathy. Thus, it can be concluded from our findings that the levels of Glucose, tota, total serum cholesterol, triglycerides, total lipids, VLDL and LDL-cholesterol which are actually raised in diabetic cardiomyopathy can be lowered with ethanolic extract of *Cardia dichotma*. Level of body wt; total protein, serum albumin, serum globulin, and HDL decreased in diabetes cardiomyopathy can be increased with ethanolic extract of *Cardia dichotma.* Two doses were given to chosen animal model and dose at 200mg/kg has shown its significant effect. Further studies both on the extract and/or its chemical constituents are needed to pinpoint the findings. This report may serve as a footstep on this aspect and conclusion.

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