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Pharmacological Screening Of *Chrozophora Tinctoria* Leaves Extract As Antidiabetic Effect In Rat

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ABSTRACT

Objective: The present study was carried out to evaluate the pharmacological screening of *Chrozophora tinctoria* leaves extract as antidiabetic effect in rat.

Method: The method is used to evaluate pharmacological potential of aqueous leaves extract as antidiabetic effect in rat. In this model, streptozotocin(60mg/kg b.w.) induced rats for one day through intraperitoneal administration, the effect of *Chrozophoratinctoria* at different dose level that is higher dose (100mg/kg b.w. p.o.) and lower dose (50mg/kg b.w. p.o.). From the study observed that *Chrozophoratinctoria* significantly protect the destruction of β-cells of pancreas from streptozotocin induced diabetes in rats. Streptozotocin induced ruputered and destruction of islet of langerhens and acini, destruction of intralobular duct and interlobular duct, and necrosis in parts of pancreas was found to be reduced in the groups receiving *Chrozophoratinctoria* along with streptozotoc in. *Chrozphoratinctoria* also normalized the streptozotocin induced diabetes decrease the blood glucose serum insulin, protein and uric acid levels. This is also evidenced by the histopathological studies.

Result and discussion: The ant diabetic activity of *Chrozophora tinctoria* was determined by having comparsion between diseased group and treatment group with reference to standard group on the basis of improvement in elevated levels of biomarkers such as blood glucose ($97.8\pm5.7^{**}$), serum insulin($1.48\pm0.36^{**}$), protein($6.4\pm0.36^{**}$), uric acid($2.04\pm0.27^{**}$), triglyceride($135.2\pm3.20^{**}$), total cholesterol($02.5\pm2.6^{**}$), SGOT($88.81\pm6.90^{**}$), SGPT($67.8\pm10.28^{**}$), HDL($51.76\pm7.92^{**}$) and LDL($80.68\pm10.62^{**}$) decrease the level of TBARS($258.93\pm7.85^{**}$) and increase the level of SOD ($73.82\pm10.60^{**}$), Catalase($16.47\pm0.42^{**}$) and GSH (0.47 ± 0.21^{ns}). At the last it was observed that high dose(100mg/kg) of *Chrozophora tinctoria* was more significantly effective as compared to the low dose (50mg/kg) on the basis of evaluation of overall biological parameter and histopathological activities.

Keywords:*Diabetes, Streptozotocin, Chrozophoratinctoria, antioxidant, Blood glucose level, Serum insulin, Uric acid*

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INTRODUCTION

Diabetes mellitus is a group of diseases characterized by hyperglycaemia resulting from reduced insulin production, abnormal insulin action or both. Although most cases of diabetes can be categorized as either type 1 (T1DM) or type 2 (T2DM), there are many less common aetiologies, ranging from single-gene disorders to rare syndromes and acquired causes[1].The abnormal high blood sugar usually associated with diabetes with long-standing harm, dysfunction, and failure of special organs, mostly the eyes, kidneys, nerves, heart, and blood vessels. It might show the way to morbidity and mortality surrounded by these diabetic populations[2].

It is an different assembly of disorder make a distinction by fasting hyperglycemia(empty stomach), postprandial hyperglycemia(after taking a meal), glucosuria, high level of fat(hyperlipidemia), and occasionally ketonemia[3]. Diabetes mellitus may present with characteristic symptoms contain passing abnormally huge amounts of urine(polyuria),excessive thrist (polydipsia), weight loss, occasionally with increase appetite (polyphagia), and blurred vision(decreased clarity)[4]. Acute, life-threatening consequences of unrestrained diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome. Diabetes complications are separated into micro vascular (due to damage to small blood vessels) and macro vascular (due to damage to larger blood vessels). Micro vascular complications consist of damage to eyes (retinopathy) leading to blindness, kidneys (nephropathy) leading to renal failure

and to nerves (neuropathy) leading to inability and diabetic foot disorders (which include severe infections leading to amputation)[5].

Many evidences indicate that the complications seen in diabetes as a result of free radicals production. However, the uncontrolled diabetes have species increased of auto oxidation of glycosylated proteins, damage membrane induction, cellular lipids proteins oxidation and activation of the sorbitol pathway[6]. Production of free radicals increases with Hyperglycemia leading to liver injuries related to carbohydrate metabolism disorder[7,8]. These injuries are represented by cellular necrosis due to increased oxidation and lipid accumulation in the hepatocytes[9]. Many complications are caused by defects in the body antioxidant defense systems[10], oxidative stress, DNA damage and cell death[11]. Natural antioxidants from plants repair these damages, and may be safe, an effective and economical alternative therapy for diabetes protection[12].

Medicinal plants have been largely used in treating various diseases. *Chrozophoratinctoria* is an annual prostrate herb common of dry waste places on sandy clay loam and flowering in April to September, it is belonging Euphrobiaceae[13]. It is known as dyer's-croton, giradol or turnsole. the plant is used to treat warts, emetic, cathartic and fever. Root ashes are given to children for cough. The seeds are purgative or cathartic, while its bark is used for tanning and dyeing[14]. Analysis of *Chrozophoratinctoria* stems, leaves, and seeds showed the occurrence of tannins, flavonoids, phenolics, alkaloids, glycosides, reducing sugars, chlorides, and sulfates were detected in samples from both habitats[15]. This report is the first on investigating the antidiabetic effect in STZ-induced diabetic rats by examining the protective effects of varying doses of aqueous *Chrozophoratinctoria* extract on pancreas and histopathological changes possibly occurring in diabetic experimental rats which may serve to fill the knowledge gap about the possible effective treatment of DM.

MATERIALS AND METHODS

Collection and authentication of plant material:

The herbal plant *Chrozophoratinctoria* is purchased from the local market of Khari Bauli, authenticated by the Botanical Survey of India, Northern regional center 192, Kaulagarh Road, Dehradun 248195 and authentication no. BSI/NRC/TECH.(Ident.)/2012-13/755.

Extraction of Leaves:

The fresh shade dried leaves of *Chrozophoratinctoria*(1000 g) were collected individually and powdered for the extraction. 150 gm of powder soaked into 1 liter of distilled water in 4 beakers kept for 48 hrs at the room temperature and add 10 ml of chloroform. The obtained dark brown extract was filtered with cheesecloth to obtain a clear solution. After extraction, it was dried to formulate powder form and stored in a well tight closed container. The acquired powdered drug was subjected to the study of pharmacological activity on rats.

Experimental Animals

Male Wistar rats (3-4 months old) will be selected for the study and followed by acclimatization for 2 weeks by maintaining standard environmental conditions and fed with standard pellet diet & water. The experiment were conducted with approval no. HIPER/IAEC/28/18/10 by IAEC (UP).The experiment will be carried out by the CPCSEA guidelines and Institutional animal ethical clearance. Then all animals will be randomized into 5 groups with 6 animals in each group.

Experimental Design

Animals Experimental Design Twenty-five male wistar rats were randomly divided into five groups and placed in cages according to the groups, containing 6 rats per group. Group I, considered as the normal control, given standard food and water for a period of 28 days; Group II, served as the diabetic control, given Streptozotocin 60mg/kg b.w. as a single dose; Group III, received Streptozotocin (60mg/kg b.w.) and given leaves aqueous extract of Chrozophoratinctoria 50mg/kg b.w. administrated orally every day for a period of 28 days; Group IV, received Streptozotocin (60mg/kg b.w.) and given leaves aqueous extract of Chrozophoratinctoria 100mg/kg b.w. administrated orally every day for a period of 28 days. Group V, received Glibenclamide (5mg/kg b.w.) administrated orally every day for a period of 28 days followed by receiving standard food and water for diabetic control and the treated diabetic rats. After 28 days, the rats were fasted for 12-hours and sacrificed by inhalation chloroform. Blood samples were obtained by means of retro orbital, serum sample was separated and for estimation glucose, serum insulin, Protein, Cholesterol, Triglyceride, Uric acid level, SGPT, SGOT, HDL, and LDL, tissues were homogenated for antioxidant study and pancreas tissue were collected for histopathology study.

Estimation of biochemical parameter

The biochemical parameters like blood glucose level, serum insulin, protein, uric acid, triglyceride, cholesterol, HDL, LDL, SGOT, SGPT and antioxidant were estimated as per the standard procedure. The blood glucose was

estimation using a D-Glucose GOD-POD Colorimetric Assay Kit (*GOD/POD method*), Serum insulin were determined by using Insulin ELISA kit(*ELISA method*), protein by Total Protein Assay Kit (*Biuret method*), uric acid by Urea (BUN) Colorimetric Assay kit (*Uricase Method*), total cholesterol, HDL and LDL by Cholesterol test kit (*Enzymatic method*), triglyceride by Triglyceride Enzymatic test kit (*Enzymatic-colorimetric end-point method*), SGPT by Colorimetric Aspartate Aminotransferase (AST) Assay Kit and SGOT by Alanine Aminotransferase (ALT or SGPT), Assay Kit[16].

Estimation of antioxidant parameter

The antioxiadant parameter like SOD(Mc Cord and Fridovich method), CAT (Beer and Sizer method) and Reduced Glutathione (GSH) (Moron *et al.Method*)[17].

Preparation of tissue homogenate:

Animal after sacrificed, the pancreas were washed thoroughly and rinsed with ice. 10% of homogenate was prepared in 0.05M phosphate buffer (pH 7) using a homogenizer at 4°C. A part of this homogenate was used for the determination of glutathione reduced (GSH). The rest of the homogenate was centrifuged at 10,000 rpm for 20 minutes for removing the cell debris, unbroken cells, nuclei, erythrocytes, and mitochondria. The supernatant was used for the determination of superoxide dismutase (SOD), thiobarbituric acid reactive substance(TBARS) and catalase (CAT). [18]

Histopathological examination:

Pancreas tissues were obtained on the last day of an experiment after scarification of animals immediately store in 10% neutral formalin solution and washed with 70% of ethanol for histopathological examination. Tissues were then placed in small metal caskets, stirred by a magnetic stirrer, dehydrated using alcohol series from 70% to 100% alcohol and embedded in paraffin using an embedding machine. Paraffin blocks were sectioned of 5µm in thickness using a rotatory ultra-microtome. The slides were observed under an light microscope at 10x and 40x after being stained with hematoxylin and eosin (H & E stain) dyes and mounted[19].

Statistical analysis:

All data were expressed as mean ± SD. Data were statistically analyzed using one-way analysis of variance (ANOVA) using graph pad prism software 8. Bonferroni test was applied for the detection of significance between different groups for the control and diseased group. $P<0.001$, $P<0.05$ are considered as extremely significant and moderate significant from control. $P>0.05$ is considered as non-significant to control.

RESULTS AND DISCUSSION

1. Phytochemical screening of plant:

Table 1 Phytochemical screening of plant

S.No.	**Active compound**	**Leaves**
1.	Tannin	+
2.	Saponin	+
3.	Glycoside (anthraquinone)	+
4.	Terpenoid	+
5.	Flavonoid	+
6.	Phenolic	-
7.	Cumarin	-

2 Estimation of *Chrozophoratinctoria* on Body weight:

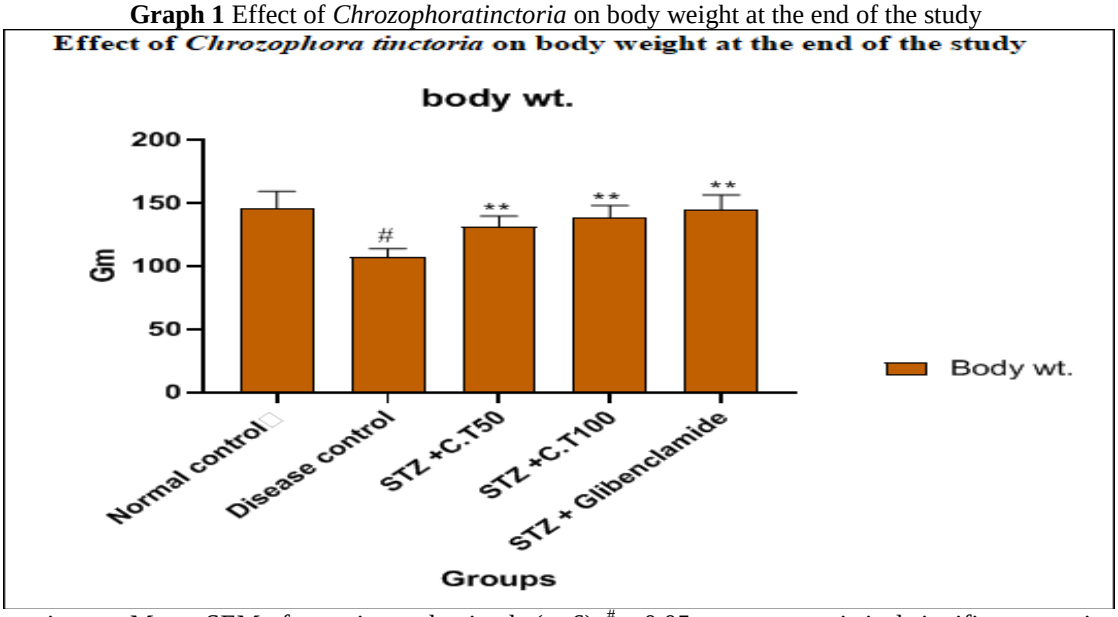
The average body weight of animal in streptozotocin treated group shows a slightly decrease in body weight (107.01±06.83g) compared with treatment as well as normal control group (145g) while the *Chrozophoratinctoria* and standard drug treated animals showed a little increased in weigh (144g) as compared to streptozotocin treated group of animal.

Table 2 Effect of aqueous extract of leaves *Chrozophoratinctoria*on body weight in rats.

Group	**Body weight (g)**
Normal Control	145.50±13.68
Disease Control (STZ)	107.01±06.83$^{\#}$
Diabetic + C.T.(50)	131.38±08.36**
Diabetic + C.T (100)	138.21±09.92**
Diabetic + Glibenclamide	144.30±12.15**

Values are given as Mean±SEM of experimental animals (n=6), #p<0.05 represent statistical significance against normal control, **p<0.05 represent statistical significant against disease control.

Graph 1 Effect of *Chrozophoratinctoria* on body weight at the end of the study



Values are given as Mean±SEM of experimental animals (n=6), #p<0.05 represent statistical significance against normal control, **p<0.05 and **p<0.01 represent statistical significant against disease control, ns= non significant.

3 Estimation of Chrozophoratinctoria on blood glucose level

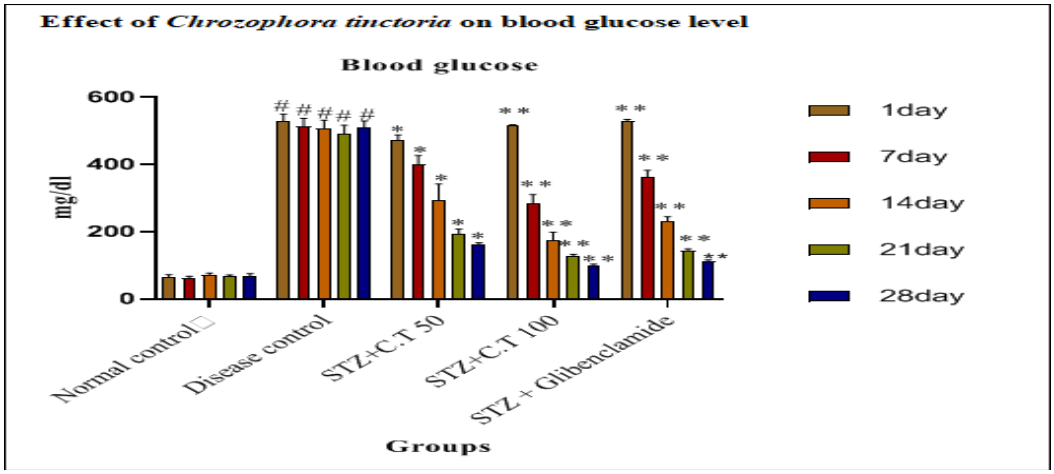
Blood glucose level gradually increases from 1st day, 7th day and 14th day in the disease control group (p<0.0001) as compared with a normal control group. The blood glucose level was observed to significantly decrease on the administration of test drug *Chrozophoratinctoria* (50mg) (*p 0.01) and (100mg) (**p 0.001) as compared to a disease control group. Thus it found that rats showed dose-dependent behavior.

Table 3 Effect of aqueous extract of leaves *Chrozophoratinctoria*on 28 days treatment on blood glucose level in rats.

Group	1 day	7 day	14 day	21 day	28 day
NC	65.0±7.07	62.7±4.61	71.3±5.37	68.0±3.65	68.7±6.32
DC	528.0±21.6#	512.2±24.46#	506.8±25.18#	491.0±25.01#	509.0±20.2#
Diabetic+ CT 50	471.7±15.88*	399.0±27.24*	292.8±48.95*	193.5±24.54*	161.3±12.9*
Diabetic+ CT 100	514.5±13.67**	285.3±18.7**	174.0±14.46**	126.7±5.96**	97.8±5.79**
Diabetic+GBM 5	527.5±13.81**	362.7±8.48**	231.8±5.89**	143.2±5.22**	113.3±3.08**

Values are given as Mean±SEM of experimental animals (n=6),#p<0.05 represent statistical significance against normal control,**p<0.05 represent statistiacal significant against disease control

Graph 2 Effect of aqueous extract of leaves *Chrozophoratinctoria*on 28 days treatment on blood glucose level in rats.



Values are given as Mean±SEM of experimental animals (n=6), #p<0.05 represent statistical significance against nomal control, **p<0.05 and *p<0.01 represent statistical significant against disease control, ns= non significant.

4 Estimation of *Chrozophoratinctoria* on protein, uric acid, and insulin in a serum sample

The estimation of the serum sample shows that the protein (3.3±0.50)g/dl and insulin level (0.09±0.39)mg/dl were significant (#p<0.05) decrease in disease control. On other hand the level of uric acid (4.13±0.30) mg/dl were significantly increasing. The prepared *Chrozophoratinctoria* extract at doses of 50mg and 100mg treatment and standard (glibenclamide) drug significantly (*p<0.01) increase the elevated levels of protein and insulin while decreasing the level of uric acid.

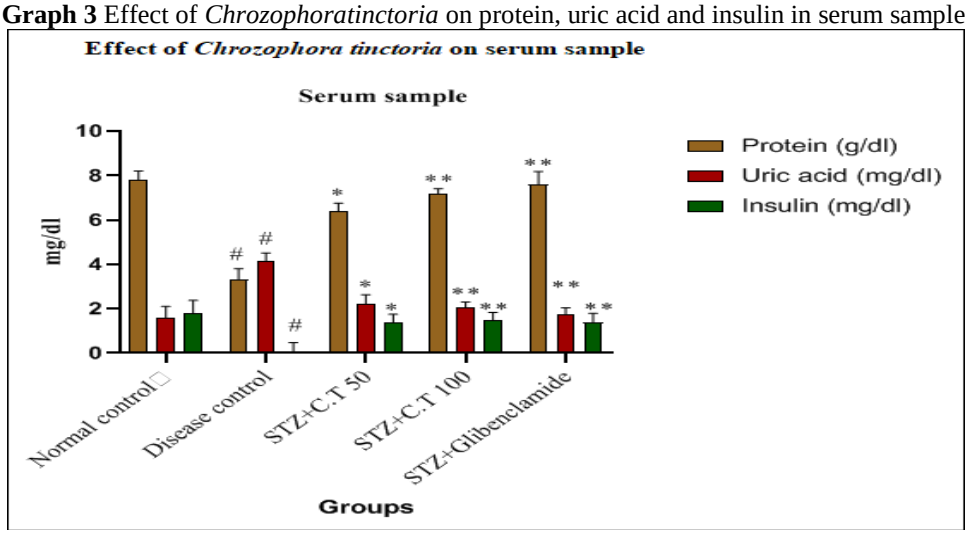
Table: 4 Effect of *Chrozophoratinctoria* leaves extract on protein, uric acid, and insulin in the serum sample

Values are given as Mean±SEM of experimental animals (n=6),#p<0.05 represent statistical significance against normal

Group	Protein (g/dl)	Uric acid (mg/dl)	Insulin (mlU/L)
Normal Control	7.8±0.41	1.60±0.50	1.80±0.58
Disease Control (STZ)	3.3±0.50#	4.13±0.30#	0.09±0.39#
Diabetic + C.T.(50mg)	6.4±0.36**	2.22±0.42**	1.36±0.39**
Diabetic + C.T(100mg)	7.2±0.21**	2.04±0.27**	1.48±0.36**
Diabetic+ Glibenclamide(5mg)	7.6±0.59**	1.74±0.31**	1.39±0.41**

control,** p<0.05 represent statistical significant against disease control.

Graph 3 Effect of *Chrozophoratinctoria* on protein, uric acid and insulin in serum sample



Values are given as Mean±SEM of experimental animals (n=6), $^{\#}p<0.05$ represent statistical significance against nomal control, $^{**}p<0.05$ and $^{*}p<0.01$ represent statistical significant against disease control, ns= non significant.

5 Estimation of *Chrozophoratinctoria* on a biological parameter at the end of the study

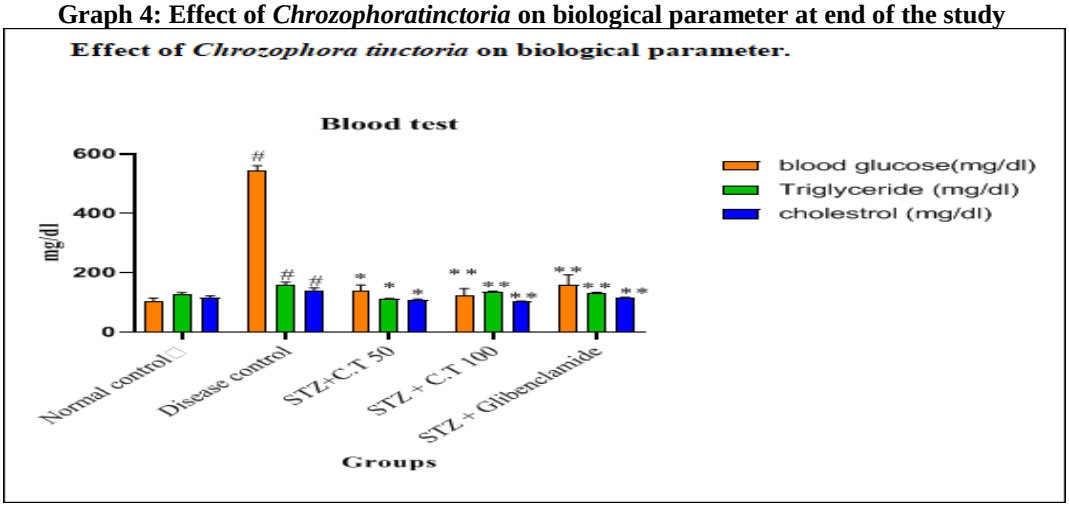
The estimation of blood sample shows that the blood glucose (542.2±18.79)mg/dl triglyceride (159.5±9.20)mg/dl and total cholesterol level (140.5±8.18) were significantly ($^{\#}p<0.05$) increases in disease control. The prepared *Chrozophoratinctoria* extract at doses of 50mg and 100mg treatment and standard (glibenclamide) drug significantly ($^{*}p<0.01$) reduces the elevated levels of blood glucose, triglyceride, and total cholesterol level

Table: 5 Effect of aqueous extract of leaves *Chrozophoratinctoria* on a biological parameter at the end of the study

Group	Blood glucose (mg/dl)	Triglyceride (mg/dl)	Total cholesterol (mg/dl)
Normal Control	102.8 ±12.29	125.7±8.22	116.8±5.54
Disease Control (STZ)	542.2±18.79$^{\#}$	159.5±9.20$^{\#}$	140.5±8.18$^{\#}$
Diabetic + C.T.(50mg)	137.7±21.7*	111.2±3.2*	108.0±3.51*
Diabetic + C.T (100mg)	125.3±21.7**	135.2±3.2**	102.5±2.6**
Diabetic+Glibenclamide (5mg)	159.0±34.03**	131.7±2.36**	114.5±2.63**

Values are given as Mean±SEM of experimental animals (n=6),$^{\#}p<0.0001$ represent statistical significance against normal control,$^{**}p<0.05$ and $^{*}p<0.01$ represent statistiacal significant against disease control.

Graph 4: Effect of *Chrozophoratinctoria* on biological parameter at end of the study



Values are given as Mean±SEM of experimental animals (n=6), ns= non significant, $^{\#}p<0.05$ represent statistical significance against nomal control,$^{**}p<0.05$ and $^{*}p<0.01$ represent statistical significant against disease control.

6 Estimation *Chrozophoratinctoria* on SGOT, SGPT, HDL, and LDL in rats

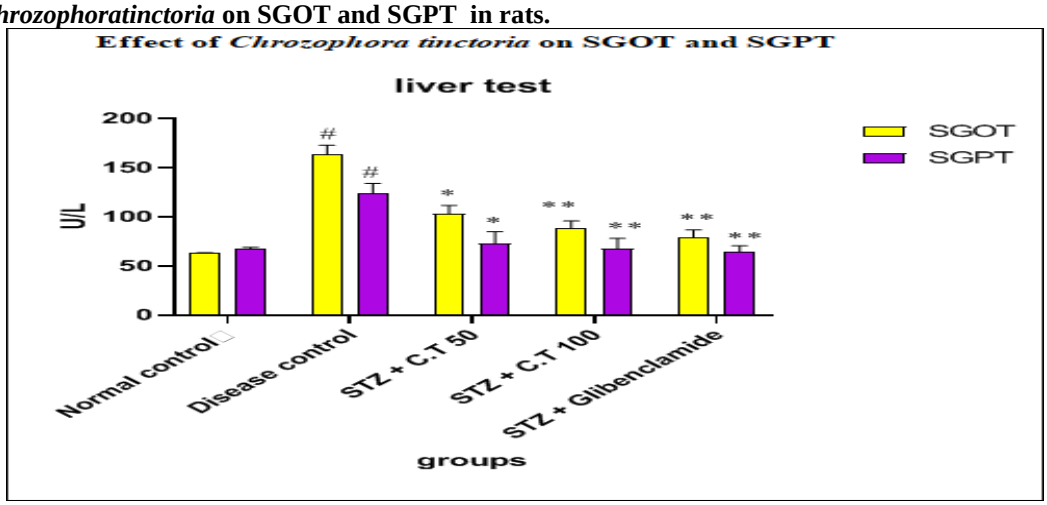
The estimation of SGOT, SGPT, HDL, and LDL shows that the SGOT(163.01±9.72)U/l and SGPT (123.39±10.68)U/l and LDL level (249.30±11.9) were significantly ($^{\#}p<0.05$) increases in disease control. On other hand the level HDL (36.30±10.29) mg/dl were significantly decreasing. The prepared *Chrozophoratinctoria* extract at doses of 50mg and 100mg treatment and standard (glibenclamide) drug significantly ($^{*}p<0.01$) reduces the elevated levels of SGOT, SGPT, and LDL while increases in the level of HDL.

Table: 6 Effect of aqueous extract of leaves *Chrozophoratinctoria*on SGOT, SGPT, HDL and LDL in rats.

Group	SGOT	SGPT	HDL	LDL
Normal control	62.96±0.60	67.62±1.39	82.2±0.82	50.08±1.98
Disease control	163.01±9.72$^{\#}$	123.39±10.68$^{\#}$	36.30±10.29$^{\#}$	249.30±11.9$^{\#}$
Diabetic +C.T(50mg)	103.28±8.25*	72.85±12.05*	41.98±10.29^{ns}	103.99±7.45*
Diabetic+C.T (100mg)	88.81±6.90**	67.8±10.28**	51.76±7.92**	80.68±10.62**
Diabetic+ Glibenclamide	79.31±7.30**	64.5±6.10**	56.15±5.35**	75.77±9.12**

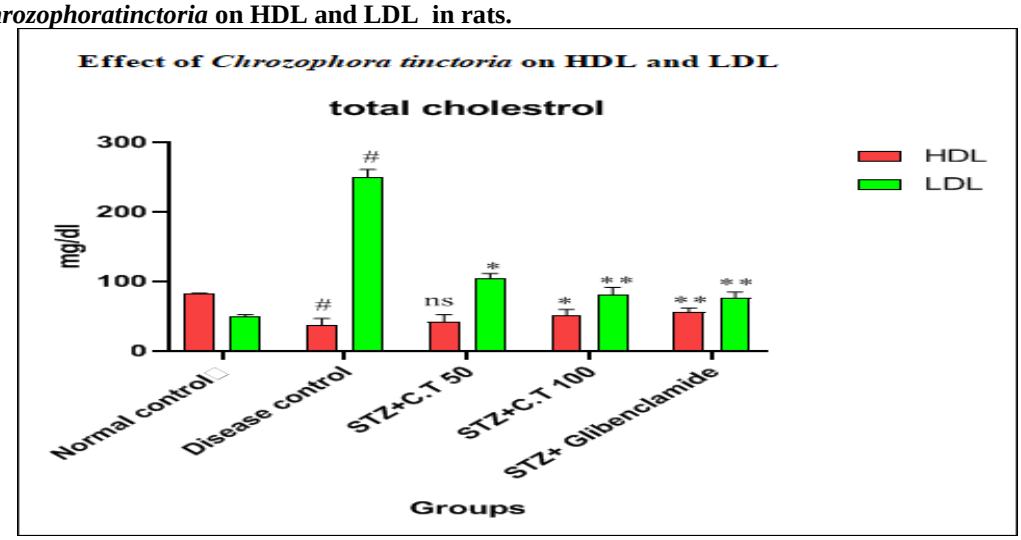
Values are given as Mean±SEM of experimental animals (n=6), #p<0.05 represent statistical significance against normal control, **p<0.05 represent statistical significant against disease control.

Effect of *Chrozophoratinctoria* on SGOT and SGPT in rats.



Values are given as Mean±SEM of experimental animals (n=6), ns= non significant
#p<0.05 represent statistical significance against nomal control, **p<0.05 and *p<0.01 represent statistical significant against disease control.

Effect of *Chrozophoratinctoria* on HDL and LDL in rats.



Values are given as Mean±SEM of experimental animals (n=6), #p<0.05 represent statistical significance against nomal control,**p<0.05 and *p<0.01 represent statistical significant against disease control, ns= non significant.

7 Estimation of *Chrozophoratinctoria* on antioxidant in rats.

The estimation of antioxidant shows that the SOD (53.15±8.32)U/mg protein,GSH(0.42±1.21)U/mg protein and CAT (22.19±8.21)were significantly (#p<0.05) decrease in disease control. On other hand the level of TBARS(322.24±5.08) U/mg protein were significantly increasing. The prepared *Chrozophoratinctoria* extract at doses of 50mg and 100mg treatment and standard (glibenclamide) drug significantly (*p<0.01) increase the elevated levels of SOD, GSH and CAT while decreasing the level of TBARS.

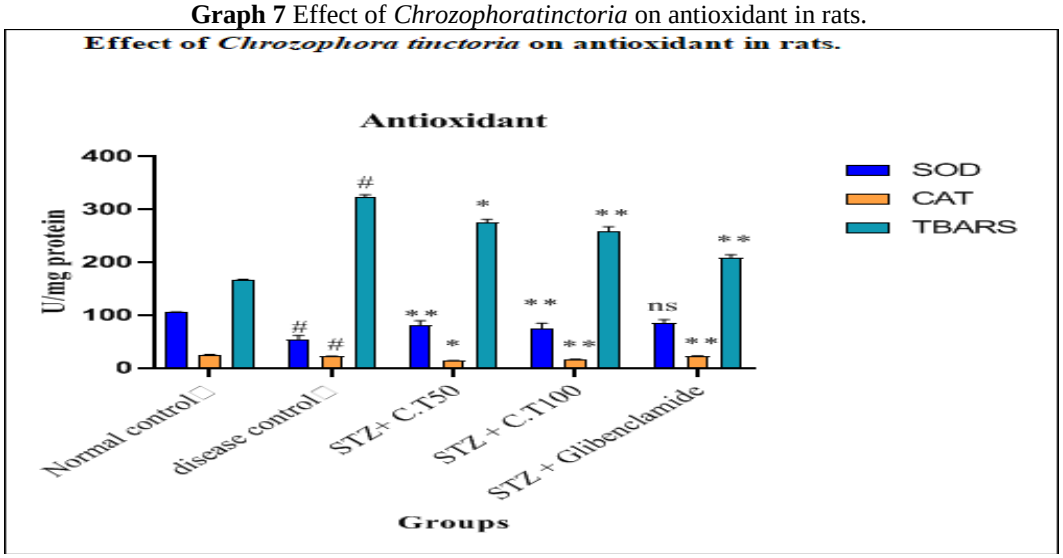
Table: 7 Effect of aqueous extract of leaves *Chrozophoratinctoria*on antioxidants in rats.

Group	SOD(U/mg protein)	GSH(U/mg protein)	CAT(U/mg protein)	TBARS(U/mg protein)
Normal control	104.72±1.42	1.57±0.01	24.96±0.60	166.60±1.20
Disease control	53.15±8.32#	0.42±1.21#	22.19±8.21#	322.24±5.08#
Diabetic+ C.T(50mg)	80.26±8.75**	0.34±0.16ns	13.50±1.50*	275.03±6.10*
Diabetic +C.T.(100mg)	73.82±10.60**	0.47±0.21ns	16.47±0.42**	258.93±7.85**

Diabetic+ Glibenclamide (5mg)	84.7±6.85^{ns}	0.74±0.10**	22.19±10.13**	207.94±6.18**

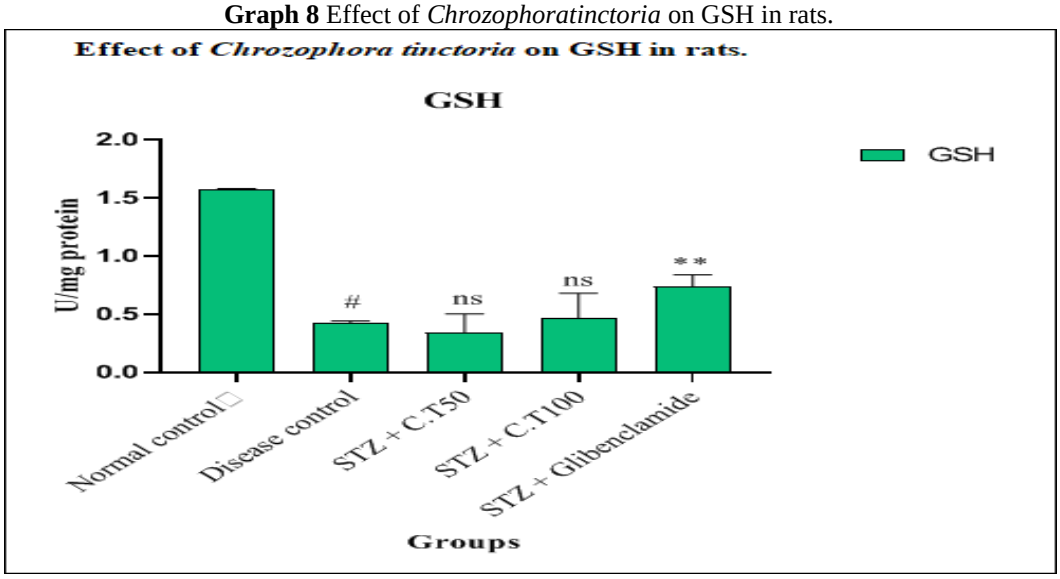
Values are given as Mean±SEM of experimental animals (n=6), $^{\#}$p<0.05 represent statistical significance against normal control, and **p<0.05 and *p<0.01 represent statistical significant against disease control.

Graph 7 Effect of *Chrozophoratinctoria* on antioxidant in rats.



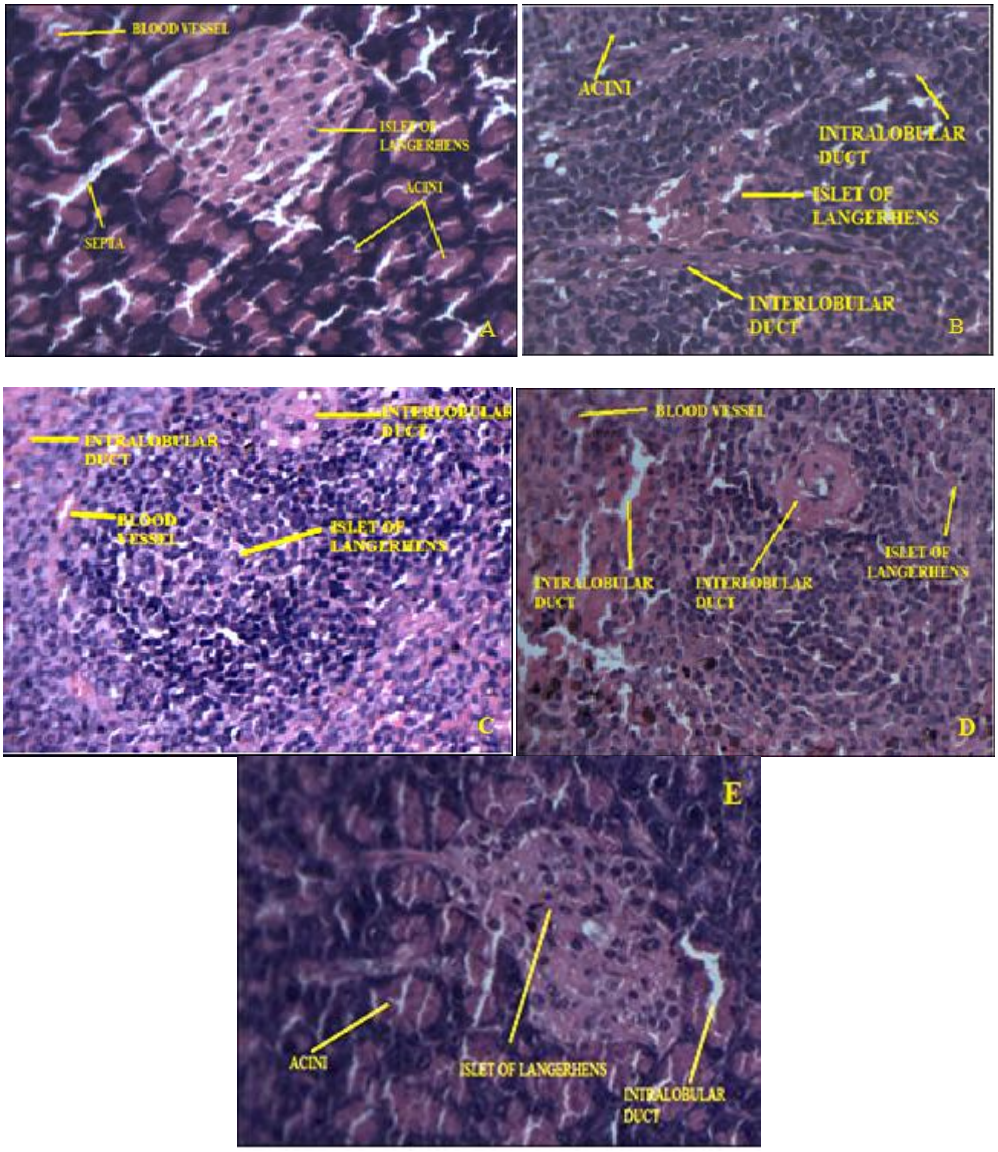
Values are given as Mean±SEM of experimental animals (n=6),$^{\#}$p<0.0001 represent statistical significance against nomal control, **p<0.001 represent statistical significant against disease control, ns= non significant.

Graph 8 Effect of *Chrozophoratinctoria* on GSH in rats.



Values are given as Mean±SEM of experimental animals (n=6), $^{\#}$p<0.05 represent statistical significance against nomal control, **p<0.05 represent statistical significant against disease control, ns= non significant

Histopathology



Pancreas sections of treated diabetic rats releave noticeable degree of islet protection against the destruction. The capability of herbal plant *Chrozophoratinctoria* to protect pancreas seems to be in proportion to increasing dose of the aqueous extract as compared to the STZ-non treated group. The section of rat liver of normal control group show normal islet of Langerhens with pancreatic acini in exocrine pancreas, both the duct intralobular duct and interlobular duct is normal in size and shape, blood vessel is also normal in shape and size in with normal septa (Fig A). While (figure B) showed the tissue section of pancreas after with (STZ). The histological results of pancreas after injection with STZ noted ruputered and destruction of islet of Langerhens and acini changes in shape and size in exocrine pancreas, destruction of intralobular duct and interlobular duct, necrosis occurs in blood vessel and the present erythrocytes inside the blood vessel spread into the septa. The pancreas sections obtained from STZ-diabetic rats treated with different doses of *Chrozophoratinctoria*(50mg/kg and 100mg/kg) showed less pathological changes and improved destruction of pancreas. In (figure C) the tissue section of pancreas after treated with extract of 50mg/kg showed the slight changes in the exocrine and endocrine pancreas. The islet of Langerhens and acini damage appears with disruption of epithelial cells. The wall of blood vessel ruptured but erythrocytes present inside the blood vessel. The thin layer of septa is seen in slide. Both ducts are destructed and ruptured. The tissue section of pancreas after treated with extract 100mg/kg showed in (figure D). The presence of less destruction and rupture islet of Langerhens with acini in exocrine pancreas. Regaining of shape and size of both the duct intralobular and interlobular duct. Regeneration of septa and wall of a blood vessel. Tissue section of pancreas treated with Glibenclamide 5mg/kg is given in (figure E). Current result of pancreas showed normal islet of langerhens. The tissue results of pancreas after treated with 100mg/kg were improved in the structured as similar to tissue structure of control group generally. Highest improvement as well as antidiabetic effect exhibited in the dose of 100mg/kg as mention in (figure D), as compared to the control group in (figure A).

CONCLUSION

This study was initiated to scientifically evaluate the claimed antidiabetic effect of *Chrozophoratinctoria* leaves extract. Diabetes is a never-ending disease caused by an absolute or relative lack of insulin and or reduces insulin action, which results in an abnormality in carbohydrates, protein and fat metabolism. Decrease physical doings, increasing flabbiness, stress and changes in food burning up have been the major cause for the increasing number of the patient. Diabetes is a "silent killer disease" it leads to some diseases and the patient remains unaware about the cause of leading problems in the body.

Streptozotocin induces diabetes, a β-cytotoxin, leads to induction of 'chemical diabetes' in a wide range of animal species including rats by selectively damaging the insulin secreting β-cells of the islet of Langerhens of the pancreas. Streptozotocin is injected through an intraperitoneal route which produces fragmentation of DNA of β-cells of the pancreas which stimulates poly ADP-ribose and depletes NAD ultimately leading to depletion of β-cells and it is evidenced by clinical symptoms such as rise in blood sugar level and hyperglycemia. The blood glucose level gradually increases from 1st, 7th, and 14th days in the disease control group as compared with the normal control group. Body weight is one of the reasons associated with metabolic regulation for diabetes.

STZ diabetic rats showed body weight loss might occur due to increase in muscle atrophy or might be due to loss of tissue proteins. The reduced level of glucose by stimulating gluconeogenesis in cells, which causes a decrease in the body weight, as well as insulin deficiency inhibits all anabolic processes and promotes catabolic processes, generally leading reversed by the further to body weight loss, through increase of glycosuria and polyuria. Treatment with *Chrozophoratinctoria* extract improved the reduction in the body weight in diabetic rats and maintains food and water intake. The administration of the aqueous extract *Chrozophoratinctoria* to experimental diabetic rats showed improvement in body weight when compared to the non-treated group, which may be due to the protection effect of the extract or due to the bioactive constituents of *Chrozophoratinctoria* to maintain hyperglycemia. Administration of *Chrozophoratinctoria* to STZ-induced diabetic rats after 35 days showed a decrease in the plasma glucose level, perhaps by the augmenting quantity of insulin in all treated diabetic rats.

The mechanism of action of aqueous *Chrozophoratinctoria* extract is unknown, involved in utilization of glucose and regulates insulin secretion. Plasma insulin levels in STZ diabetic rats were observed decreased significantly. Whereas treated of diabetic rats with *Chrozophoratinctoria* extract shows increases in plasma insulin levels due to phytochemical such as alkaloid, flavonoid, saponin, Terpenoid and anthraquinone. These phytochemicals act in a number of diverse mechanisms to bring about their anti-diabetic effect. Some of the mechanisms involved in insulin secretion, decreases in hepatic glucose output, regulation of certain enzymes carbohydrate metabolism such as a- glucosidaseinibitors, modulation of certain regulation molecule such as PPARγ, hypolipidaemic activities, antioxidant effects, interference with some glycolytic enzymes such as phosphoenolpyruate, carboxykinase activities, enhancement glucose transporters and others[20].

Common biochemical markers to determine changes in their levels namely protein, uric acid, triglyceride, cholesterol, SGOT, SGPT, HDL, and LDL were also estimated. In this enzyme level of uric acid, triglyceride cholesterol, SGOT, SGPT, and LDL is increase while the level of protein and HDL is decreased in activity during diabetes. The administration of *Chrozophoratinctoria*(50mg and100mg) to the treated groups which show a significant decrease in levels of uric acid, triglyceride, cholesterol, SGPT, SGOT, and LDL while the increase in the level of protein and HDL as compared to non treated group.

As this plant contain phytochemical is already proved that flavonoid having an antidiabetic property which may ability to modulate some cell signaling like it inhibiting glucogenesis through inhibition of mitochondrial pyruvate transport and decrease in the cytosolic NADH/NAD redox. Decreasing of glycogen breakdown, plasma glucose levels, glycosylated haemoglobulin, mRNA and protein expression levels of gluconeogenesis genes like phosphoenol pyruvate carboxykinase and by increasing plasma insulin maybe brought out the antidiabetic effect of this drug[21].

Saponin may be responsible for decreasing the level of protein, triglyceride, SGOT, and SGPT. Saponin also increases the production of insulin and it reduces the serum lipid level by expression of fatty acid binding protein, and Glucose-6-phosphate and at same time increase the GLUT 4. The saponin is known to increase glycogen accumulation and decrease the triacylgylcerol storage in the liver. It also enhances the expression of adiponectin and PPAPγ in adipose tissues, improves insulin signaling and increases GLUT4[22].

Alkaloid was reported as a significant improvement GLUT 4, glucokinase activity and peroxisome PPARγ this include a reduction in total cholesterol and triglyceride level. The antioxidant activity was also estimated as already know that due to the induction of Streptozotocin oxidative stress increases due to the production of ROS which causes tissue damage. STZ treatment causes a significant increase in TBARS but decreases in antioxidant enzymes like Superoxidase dismutase, catalase, and glutathione peroxidase activities when compared with the normal group. So due to the

phytochemical of *Chrozphoratinctoria* decrease the level of TBARS and increase the level of superoxidase dismutase, catalase, and glutathione peroxidase[23].

Flavonoid may be help in reducing the level of TBARS as it down regulating the generation of free radicals and release of pro-inflammatory cytokinase. Normalization of the lipids and adiponectin together with alteration of the activities of glucose metaboloizing enzymes decrease in the level of thiobarbitutic acid reactive substance. Saponin maybe reduce the oxidative stress and inhibit the production of ROS due to this the level of superoxidase dismutase level increases[24,25]. Tannin having antioxidant properties so it may increase the level of superoxidase dismutase, catalase and glutathione peroxidase. The pancreas important role in the regulation of blood glucose level[26,27]. Diabetesmilletus causes change in this organ tissue, representative photo histological view from the HE-stained of the pancreas tissue section is observed. Pancreas section of STZ induced diabetic rats showed several alterations due to lack of insulin. The major alteration including the destruction of the islet of Langerhens, changes in both the duct intralobular duct and interlobular duct, destruction of septa and blood vessel. The tissue section showed the abnormal size of the islet of Langerhens with acini similar to other experimentally induced diabetic animal models. This damage is partially reversed by the *Chrozophoratinctoria* extract treatment. The pancreas tissue of the control group did not show any histological alteration the section presented typical histological organization, normal the islet of Langerhens with acini, no change in duct, normal blood vessel and septa.

In diabetic rats treated with *Chrozophoratinctoria* extract. The slight changes in the islet of Langerhen with acini, regeneration of both duct interlobular duct and intralobular duct and regaining of blood vessels. Further histological studies are necessary to indicate the ability of *Chrozophoratinctoria* as an antidiabetic effect. In the present study, histopathological findings also aid the protective potential of *Chrozophoratinctoria* aqueous extract to stimulate the activity of insulin secretion during treated diabetes.

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