

To Evaluate the Antidiabetic and Rejuvenating Capability of Tissues on Alloxan Induced Diabetic Rats under the Effect of Ethanolic Leaf Extract of *Coriandrum sativum*: A Histopathological Study

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ABSTRACT

Objective: To evaluate the antidiabetic and rejuvenating capability of tissues on alloxan induced diabetic rats under the effect of ethanolic leaf extract of *Coriandrum sativum*.

Methods: Diabetic model was prepared by administration of alloxan monohydrate (150 mg/kg i.p). The ethanolic leaf extracts of *Coriandrum sativum* at a dose of 200 and 400 mg/kg of body weight were administered to diabetic induced groups for a period of 28 days. The effect of ethanolic leaf extract of *Coriandrum sativum* leaf extract on serum blood glucose, insulin, lipase, α -amylase and LDH as well as kidney function test [urea, uric acid, albumin, protein and creatinine] were measured in the alloxan induced diabetic rats. **Results:** In the acute toxicity study, ethanolic leaf extract of *Coriandrum sativum* leaf was non-toxic at 2 000 mg/kg in rats. The increased insulin level, albumin and protein level, decreased blood glucose and other biochemical parameters level were observed in diabetic rats treated with both doses of ethanol extract of *Coriandrum sativum* leaf compared to diabetic control rats. In Histopathological study were revealed toward normal. **Conclusion:** Ethanolic extract of *Coriandrum sativum* leaf possesses significant antidiabetic and rejuvenating capability of tissues.

Key words: Alloxan, Wistar Rats, Pancreas and Kidney Tissue, *Coriandrum sativum*.

INTRODUCTION

Diabetes mellitus is chronic disorders which are associated with many complications such as diabetic keto-acidosis, cardiovascular problems, kidney failure, eye damage, hyperosmolar coma and foot ulcers. These conditions develop due to abnormalities in carbohydrate metabolism and insulin synthesis furthermore resulting in high blood sugar with symptoms such as thirst and hungry, glycosuria, polyuria.¹ According to the World Health Organization has predicted that the number of diabetic patients will increase by the year 2025 with the current number 150 million to 300 million which is carefully thought about as the silent widespread disease of 21st centuries² However, the level of free radical and reactive oxygen species are increased, also enhanced lipid peroxidation, damage to DNA and protein degradation in diabetic patients. In type 1 diabetes, ROS are involved in β -cell dysfunction initiated.³ In type 2 diabetes, ROS is activated β -cell apoptotic pathways, impair insulin synthesis and also accord to insulin resistance.⁴ Having great knowledge about management of diabetes, the number of patient diabetes are increasing tenacious due to multiple defects in pathophysiology.⁵ There are many oral hypoglycemic agents have been used for treatment

of diabetes in which some are biguanides and meglitinides, sulfonylureas, thiazolidinediones, and α -glucosidase inhibitors used for controlling of diabetes. In this study Metformin is used which is a biguanide anti-hyperglycaemic agent and used for controlling of blood glucose level without causing hypoglycemia.⁶ Today's focus on the diabetes is controlling not only to keep blood glucose, lipid levels within a normal range but also to prevent related complication, improve patient satisfaction and quality of life.⁷⁻⁸

People were using herbal medicines from ancient time whenever there were no modern medicines and no information about the cellular and molecular function of the body.⁹ Though medicinal plants have been used in the treatment of diabetes from long times, but its acceptance and application in modern medicine need time.¹⁰ However, this alternative medicine is too attractive for people.¹¹⁻¹³ A large number of pharmacological researches have reported on the anti-diabetic effects of medicinal plants, which resulted into an increase as the number of people who use these natural compounds to control their disease.^{14,15} Before the inven-

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tion of insulin and blood glucose lowering drugs, the medicinal herbs were used for treatment of diabetes and its related complications. According to ethnobotanical data more than 1200 medicinal plants have been shown to possess anti-diabetic activities.¹⁶⁻¹⁷ In present experiment, *Coriandrum sativum* leaves were used for their antidiabetic efficacy. *Coriandrum sativum* (family: Umbelliferae) is an herb which is widely found across the world and cultivated for its nutritional value. Phytochemical analysis of *C. sativum* have contain DPPH radical scavenging activity, lipoxygenase inhibition, phospholipid peroxidation inhibition, iron chelating activity, hydroxyl radical scavenging activity, superoxide dismutation, glutathione reduction and anti-lipid peroxidation. Extraction of *C. sativum* contain a huge amount of total phenolic, tannin, saponin, pyrogallol, caffeic acid, glycitin.¹⁸ It was shown that coriander extracts have phenolic compounds with anti-oxidative activity.¹⁹ Coriander has been stated that great pharmacological affects such as anti-peroxidative,²⁰ antihyperglycaemic,²¹ hypolipidemic²² antioxidant.²³

MATERIALS AND METHODS

Identification and preparation of *Coriandrum sativum* extract

Coriandrum sativum leaf was obtained from Allahabad district in Uttar Pradesh where it is called dhaniya. The plant was authenticated by Dr. S.L. Gupta, scientist-E & Head, Botanical survey of India, Allahabad (BSI/CRC/BS-2/2014-2015). The leaf was washed and dried without exposure of sun light and. The leaf was then pulverized into powder using a grinder. It was kept in 70% ethanol for four days. The resulted extract was filtered with Whatman filter paper no-1 and concentrated by soxhlet apparatus for 12 hr at 30° C. Ethanol was evaporated using rotary evaporator under reduced pressure and low temperature it is stored in air tight containers at 10°c

Induction of diabetes mellitus

The animals were allowed to acclimatize for one-two weeks and then diabetic models were made by chemically induced in the rats using freshly prepared solution of alloxan monohydrate (dissolved in 0.9 % Na Cl) at a dose of 150 mg/kg body weight injected by intraperitoneally. After alloxan induction, rats with hyperglycemia were made likely by tail vein blood glucose level with the help of glucometer. The concentration of glucose level (>250 mg/dl) was considered in the experiment.

Experimental design for animal study and Ethical issues

Animals were kept under standard laboratory conditions (25 ± 30°C, 12 h light/dark cycle) and had free access to food and clean tap water *ad libitum* for 28 days of experimental period. The animal experiments were maintained in accordance with the Organization for Economic Cooperation and Development (Organisation for Economic Co-operation and Development) guidelines.²⁴ All the procedures were in accordance with the Institutional Animal Ethics Committee Guidelines (UIP/IAEC/APRIL-2015/08). The experimental study was conducted on five groups of animals each with six Wistar albino rats were randomly allocated to each of the five groups.

The groups were treated as follows:

Group I [CN]- consisted of normal rats, orally given water and food.

Group II [DM]- consisted of diabetic rats were received alloxan (150 mg/kg b.w.) by intraperitoneally injection.

Group III [DM+CS200] consisted of diabetic rats orally given *Corriandrum sativum* ethanolic leaf extract by gavages (mg/kg b.w.) once daily for 28 days

Group IV [DM+CS400] consisted of diabetic rats orally given *Corriandrum sativum* ethanolic leaf extract by gavages' (400 mg/kg b.w.) once daily for 28 days

Group V [DM+MET200] consisted of diabetic rats orally given standard drug Metformin (200 mg/kg b.w.) once daily for 28 days

Collection of blood sample and Measurement of biochemical parameters

After the 28 days, animals were sacrificed and blood was collected by the orbital sinus puncture method of Herck, *et al.*, 1998. Blood was collected in a dried centrifuged tube and allowed to clot. Blood was centrifuged at 3000 rpm for 15 min at room temperature. The serum was collected carefully and kept at -20° C until analysis biochemical analysis. Biochemical parameters KFT (urea, Uric acid, protein, albumin, and creatinine) pancreatic (Insulin, α- amylase, lipase, LDH) were estimated according to the protocol of the manual of diagnostic kits.

Histopathological Analysis of Rat Tissues following Treatment

At the completion of the experiment all groups animal sacrificed under using mild anesthesia (Diethyl ether). The kidney and pancreas were removed and fixed in a 10% solution of formaldehyde. The tissues were dehydrated in 30% to 100% absolute alcohol for 15 minutes. The tissues were cleaned in absolute alcohol with xylene (1:1) and then in pure xylene for one hour. Then after passing through a mixture of xylene and molten wax (1:1) for one hour, The fixed tissues were cleared in xylene and embedded in paraffin wax. The Blocks were made using L- moulds after making the sections (5 µm) from each of the tissues were examined using a light microscope after staining with hematoxylin and eosin.

Acute toxicity study

Acute oral toxicity was performed as per guideline described in OECD 423.²⁴ This is a stepwise procedure with the use of minimum animals for per step. According to protocol, albino rats, n=3 of either sex were used for this experiment. Before administration of extract, animals were kept fasting overnight and gave only water. After that, the ethanolic leaf extract of (CS-*Coriandrum sativum*) was given orally at the dose of 5 mg/kg b.w, 50 mg/kg b.w., 300 mg/kg b.w. and 2000 mg/kg b.w. daily for seven days and observed activity. Finally, 200 and 400 mg kg b.w. were selected for this study and acute toxicity study revealed the nontoxic nature for extract.

Statistical analysis

All results were expressed as mean ± standard deviation (n=6). A statistical analysis was analyzed by one way analysis variance (ANOVA) with the help of Dunnett's Multiple Comparison Test using graph pad In Stat version 5. At significant value is p<0.05

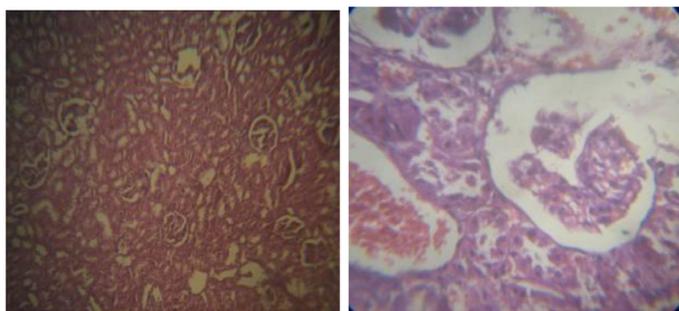
RESULTS

Serum Glucose level activity

Diabetic was made by alloxan and anti-diabetic activity of ethanolic extract of *Coriandrum sativum* leaf on alloxan induced rats were presented in Figure 1. The activity of serum glucose level (304.5±4.42 mg/dl) was significantly increased (p<0.05) in alloxan induced group compared to normal level (85.96±5.37 mg/dl). After oral administration of ethanolic extract of *Coriandrum sativum* leaf at different dose (200 and 400 mg/kg b.w) significantly decreased (p<0.05) was observed in alloxan induced diabetic rats. Which were showing prominent antidiabetic role of *Coriandrum sativum* leaf extract. However, standard drug (Metformin

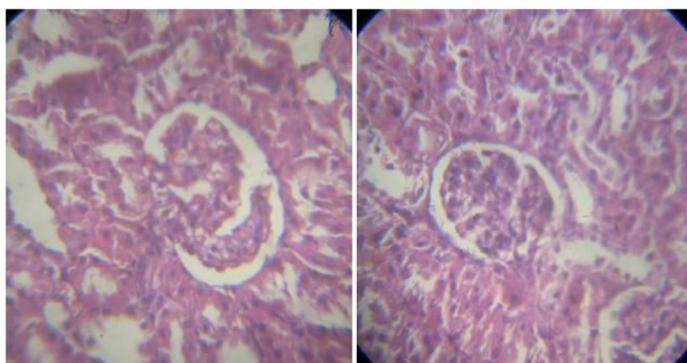
Histopathology study

Histopathology study of kidney tissue



I (normal)

II(alloxan induced)



III (DM+CS 200mg/kg bw)

IV(DM+CS 400mg/kg bw)



V (DM+metformin200mg/kg bw)

Photomicrographs of kidney (Hematoxylin and Eosin staining under a light microscope at 400_x magnification) A: control rats, B: alloxan induced rats, C: 200 mg/kg bw (CS-*Coriandrum sativum*) treated group, D: 400 mg/kg bw (CS-*Coriandrum sativum*) treated group, E: 200 mg/kg bw Metformin.

200mg /kg b.w) showing reduced the blood glucose level (179.7±4.70 mg/ dl) as compared to alloxan treated group.

Kidney function level

The level of serum albumin, creatinine, protein, urea and uric acid were presented in Figure 2 and Table 1. Significantly reduction in albumin and protein level ($p < 0.05$) were in alloxan induced diabetic rats, compared to normal group. On the other oral administration of ethanolic leaf extract of *Coriandrum sativum* on diabetic group, significantly increased. The

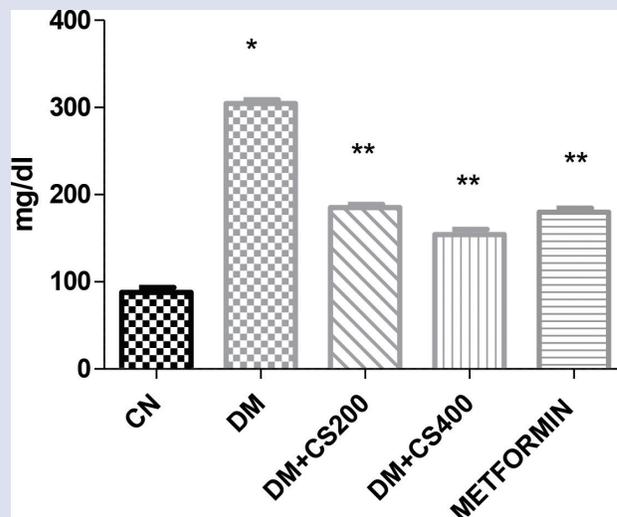


Figure 1: Effect of *coriandrum sativum* on serum glucose level in alloxan induced diabetic model. Values are statistically significant at $p < 0.05$

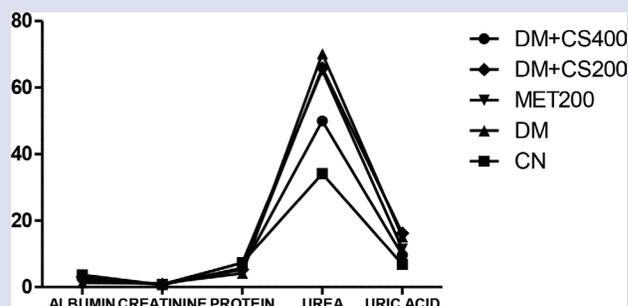


Figure 2: Effect of *coriandrum sativum* on kidney function test in alloxan induced diabetic model. Values are statistically significant at $p < 0.05$

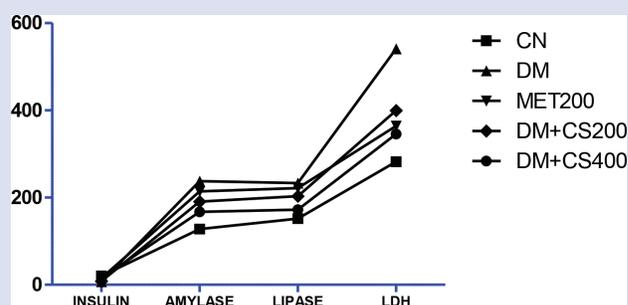


Figure 3: Effect Of *Coriandrum Sativum* On Pancreatic Test In Alloxan Induced Diabetic Model. Values are statistically significant at $p < 0.05$

Table 1: Effect of *Coriandrum sativum* leaf extracts on the serum albumin, creatinine, protein, urea and uric acid level of normal, diabetic induced and drug treated rats.

Treatment group	Albumin g/dl	Creatinine mg/dl	Protein g/dl	Urea mg/dl	Uric acid mg/dl
Group I	3.72±0.55	0.68±0.11	7.35±1.49	34.07±3.77	6.86±0.77
Group II	1.28±0.08*	1.15±0.11*	4.15±1.49*	70.17±1.60*	15.1±1.52*
Group III	1.90±0.17**	0.82±0.08**	5.27±1.68**	65.88±1.86**	10.24±2.35**
Group IV	3.11±0.13**	0.79±0.11**	5.79±1.88**	49.99±4.01**	9.60±2.06**
Group V	2.61±0.16**	0.91±0.12**	7.27±1.72**	65.16±3.53**	11.59±1.02**

Values are expressed as mean ±SD, n=6. *: comparison made between normal control to diabetic control*; P<0.05 and *: comparison made between diabetic group to drug treated groups; **: P<0.05

Table 2: Effect of *Coriandrum sativum* leaf extracts on the serum insulin, amylase lipase and LDH level of normal, diabetic induced and drug treated rats.

Treatment group	Insulin mU/L	Amylase IU/L	Lipase U/L	LDH U/L
Group I	20.19±3.20	127.6±7.25	151.70±7.89	282.30±13.78
Group II	7.04±1.77*	237.6±8.18*	233.00±5.56*	541.00±13.65*
Group III	8.40±2.19**	190.5±6.96**	203.00±6.82**	399.60±11.54**
Group IV	15.32±2.19**	167.2±5.67**	172.1±7.86**	345.80±11.17**
Group V	15.49±2.42**	214.10±8.03**	221.60±8.02**	364.10±10.91**

Values are expressed as mean ±SD, n=6. *: comparison made between normal control to diabetic control*; P<0.05 and *: comparison made between diabetic group to drug treated groups; **: P<0.05

level of urea, uric acid and creatinine were seen significantly ($p<0.05$) increased comparison with alloxan induced groups. Both the dose of *Coriandrum sativum* and Metformin treatment significantly reduced.

Pancreatic function

The level of serum insulin, lipase, α -amylase, LDH were presented in Figure 3 and Table 2. Significantly reduction in insulin level ($p<0.05$) were in alloxan induced diabetic rats, compared to normal group. On the other oral administration of ethanolic leaf extract of *Coriandrum sativum* on diabetic group, significantly increased. The level of lipase, α -amylase, LDH were seen significantly ($p<0.05$) increased comparison with alloxan induced groups. Both the dose of *Coriandrum sativum* and Metformin treatment significantly reduced.

DISCUSSION

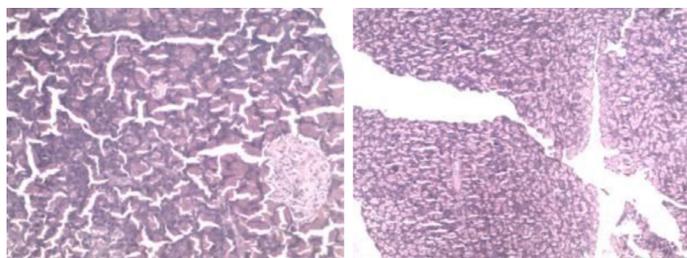
From ancient time, the medicinal plant was used for curing of various diseases across in the world. Plants are not only used as a source of nutrient but also a source of curing of disease and its ailment. Plants have a various type of phytochemical constitute which helps to cure many diseases and to boost up organ in human body.

In this study, alloxan was used to a making diabetic model. Alloxan has a capability to destruct β - cell hence increased blood glucose level. The present study revealed significant high blood glucose level in diabetic animals compared to normal animals groups. Our results are similar to the finding of several researchers using alloxan induced diabetic animals.²⁵ On the other hand, administration of ethanolic leaf extract of (*CS-Coriandrum sativum*) on alloxan induced diabetic rats for 4 weeks, the increased level of blood glucose was decreased due to the active principles present in these extracts such as Flavonoids, tannin, alkaloids saponin.²⁶ which possess the properties enhance insulin secretion, alteration

of β - cell, improvement of hepatic glutathione concentration, pancreatic lipase. Earlier reported by.²⁷ alkaloid exhibited to glycemic control. According to Jung *et al.*²⁸ suggested that alkaloid from *syzygium malaccense* and *penares schulzei* inhibited glycogen phosphorylase as well as inhibited α -glucosidase both *in vivo* and *in vitro*. Ma *et al.*²⁹ reported that Flavonoids obtained from *Morus indica* have vital role to controlling glucose level and lipid concentration, and also suggested that its connection with to improve hepatic CYP2E1 and decrease glucose activity in (*CS-Coriandrum sativum*) rats. Flavonoid are known to the regenerative properties of damaged β - cells in alloxan-induced animals and act as insulin secretagogues which are medicine for type 2 diabetes, helps secretion of insulin and maintain blood glucose level.³⁰

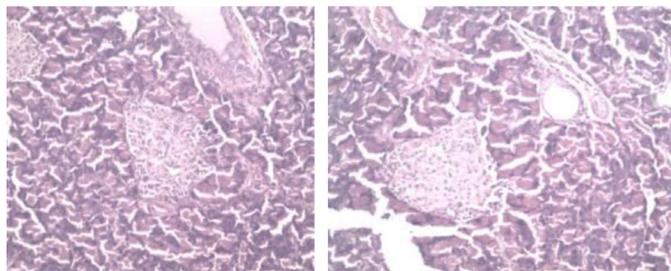
In the present experiment, the level of urea, uric acid, creatinine were significantly increased after alloxan induction. However, the level of protein, albumin were decreased. Serum urea and creatinine can be considered as a marker of renal function. These increased levels may be due to NH_3 which is converted into urea and detoxified. Urea produced in the liver and is transported into blood to kidney then finally excreted in urine continuously, whenever kidney fails to maintain NH_3 then it becomes high blood glucose level and renal failure. Studies showed that diabetic animal manifest negative nitrogen enhanced tissue proteolysis and decreased protein synthesis. It can contribute increased level of creatinine and urea indicating renal failure.³¹ Creatinine is filtered by glomerulus and it is considered as an indirect measure of glomerular filtration. Whenever, rate of glomerular filtration is diminished which lead to enhance concentrations of serum creatinine and urea. This rise indicates progression of renal disease and thus serum creatinine has more reliable indicator of renal function compared with urea for predicting the adverse outcomes.³² However, treatment of ethanolic leaf extract of C.S for 28 days, the level of kidney function revealed to normal and

Histopathology study of pancreas tissue



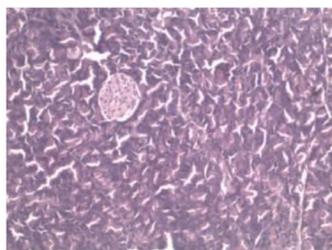
A (normal)

B(alloxan induced)



C (DM+CS 200mg/kg bw)

D (DM+CS 400mg/kg bw)



E (DM+metformin200 mg/kg bw)

Photomicrographs of pancreas (Hematoxylin and Eosin staining under a light microscope at 400_x magnification) A: control rats, B: alloxan induced rats, C:200 mg/kg bw CS treated group, D: 400 mg/kg bw CS treated group, E: 200 mg/ kg bw Metformin.

protein and albumin level were increased. Our results are similar to the finding of other researchers.³³

Pancreatic α -amylase is a prominent role of the digestive system and that hydrolysis of starch to a mixture of smaller oligosaccharide consisting of maltose and maltotriose and number of oligoglucans. These are acts with α -glucosidase and further degraded to glucose and enter into the blood stream. The degradation of starch rapidly and leads to elevation of blood glucose level.³⁴ on the other hand administration of ethanolic extract on alloxan induced diabetic rats, the increased level has significantly decreased.³⁵ Lipase is an enzyme that plays vital role in lipid metabolism which is employed in human and animal model studies.³⁶⁻³⁷ However, treatment with ethanolic leaf extract of CS on alloxan induced diabetic groups for 4 weeks, the increased level were decreased³⁸⁻³⁹ also enhance the insulin level.⁴⁰⁻⁴¹ Lactose dehydrogenase(LDH) is an enzymes that plays vital role of conversion of pyruvate to lactate and produce energy under anaerobic condition, this reaction occurs in both cytosolic and mitochondrial. The excessive pyruvate is converted into lactate for that LDH is required, therefore it becomes increased LDH activity due to less production of insulin in diabetes.⁴² Production of ROS

in alloxan induced diabetes caused tissue damage in the liver, kidney and heart, these changes can be affected the properties and function of the cell and resulting in increased synthesis of LDH.⁴³ Barbara *et al.*⁴⁴ reported that LDH levels is high in diabetic patient. Increased level of LDH activity in alloxan induced diabetic animals were directly effect on glucose metabolism and insulin secretion the β - cell of the pancreas so it may be directly responsible for insulin secretory defects in diabetes. However, treatments of ethanolic leaf extract of (CS-*Coriandrum sativum*) on these groups have exhibited to protective role.

In the present study, Histopathological examination of pancreas of alloxan induced diabetic rats revealed vacuolization, necrotic change and inflammation of lobular ducts and destruction of β -cells of the islets of langerhans. This result was similar to reported⁴⁵ although treatment of medicinal plant extract revealed a remarkable improvement. This is revealed that the plant extracts at this dose has the ability of regeneration of β -cells and increase insulin production which promotes glucose uptake and utilization by other tissue. These improvements are in β -cells due to its presence of a phytochemical constituent, have an ability to stop destruction of β -cells of the islets of Langerhans by mopping up the circulating reactive oxygen species and allowing phytochemical to enhance regenerative activities.⁴⁶ The obtained Histopathological findings are correlated with the biochemical analysis of different groups treated with extracts and supported the forgoing assumption. Earlier reported by.^{47,48} Histopathological studies recommended that increase in the number of β -cells of the islets of langerhans in diabetic rats treated with various plant extracts. On the other side, histopathology study of kidney tissues were revealed degeneration in alloxan induced tissues after treatment of extract on these group, degeneration had become normal.^{49,50}

CONCLUSION

In the current study, the administration of the ethanolic leaf extract of *Coriandrum sativum* showed significant hypoglycemic and rejuvenating activity in alloxan-induced diabetes. The extract was proved to be more potent and more effective than standard drug.

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CONFLICT OF INTEREST

All authors have none to declare

ABBREVIATION USED

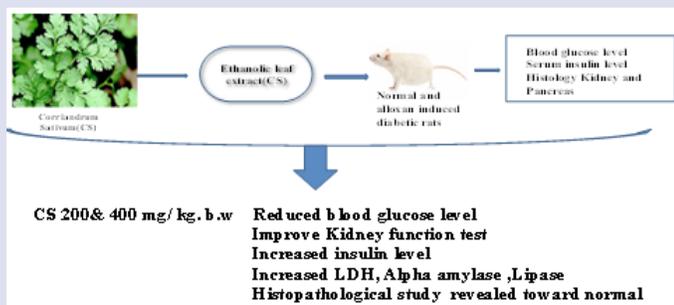
CS: *Coriandrum sativum*; STZ: Streptozotocin; OECD: Organisation for Economic; Co: operation and Development; LDH: Lactose dehydrogenase; ROS: Reactive oxygen species.

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GRAPHICAL ABSTRACT



SUMMARY

- *Coriandrum sativum* leaf extracts reduced blood glucose level in alloxan induced diabetic rats
- *Coriandrum sativum* leaf extracts increased insulin level in alloxan induced diabetic rats
- *Coriandrum sativum* leaf extracts improved kidney function test in alloxan diabetic rats.
- *Coriandrum sativum* leaf extracts improved histological study of kidney and pancreas.

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