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Original Paper

EVALUATION OF ANTIDIABETIC POTENTIAL OF HYDROALCOHOLIC LEAVES EXTRACT OF CORDIA SINENSIS PLANT

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ABSTRACT

To investigate antidiabetic activity of hydroalcoholic extract of leaves of *Cordia sinensis* in Wistar rats. Leaves extract of *Cordia sinensis* in hydroalcoholic solution were prepared by maceration method and stored. Wistar rats were made diabetic by a single dose of 'Alloxan monohydrate'. Hydroalcoholic leaves extract of *Cordia sinensis* was screened for antidiabetic activity and given to the 'Alloxan monohydrate'-induced diabetic rats at a concentration of 100 mg/kg and 200 mg/kg of body weight in different groups of 6 diabetic rats each orally once a day for 15 days. Glibenclamide is given to another group to support the result at a dose of 600µg/kg p.o of body weight orally once a day for 15 days. Blood glucose levels and body weights of rats were measured on 0, 7th, and 15th days. Oral administration of the extracts for 15th days caused a significant ($P < 0.01$) reduction in blood glucose levels in diabetic rats. The body weight of diabetic animals was also improved after daily administration of extracts. The extract also improved other altered biochemical parameters associated with diabetes. Also the changes in food intake, water intake, and weight of internal organs were also restored to normal by the prolonged effect of extract treatment.

Keyword: *Cordia sinensis,* Glibenclamide, Alloxan monohydrate

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INTRODUCTION

1.1 Diabetes mellitus It is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycaemia and glucose intolerance, as a result of lack of insulin , defective insulin action, or both. Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion, insulin action, or both. Metabolic abnormalities in carbohydrates, lipids, and proteins result from the importance of insulin as an anabolic hormone.

Low levels of insulin to achieve adequate response and/or insulin resistance of target tissues, mainly skeletal muscles, adipose tissue, and to a lesser extent, liver, at the level of insulin receptors, signal transduction system, and effector enzymes or genes are responsible for these metabolic abnormalities. Some of the diabetes patients are asymptomatic especially those with type 2 diabetes during the early years of the disease, others with marked hyperglycemia and especially in children with absolute insulin deficiency may suffer from polyuria, polydipsia, polyphagia, weight loss, and blurred vision. Uncontrolled diabetesmay lead to stupor, coma and if not treated death, due to ketoacidosis or rare from nonketotic hyperosmolar syndrome.

Damage of the β cells of the pancreas due to diffusedinjury of the pancreas can cause diabetes. This damage could be due to pancreatic carcinoma, pancreatitis, infection, pancreatectomy, and trauma[1]. Atrophy of the exocrine

pancreas leads to progressive loss of the β cells. Accumulation of fat in the pancreasor pancreatic steatosis could lead to diabetes due to decreased insulin secretion but may require a longtime before the damage to β cells occurs. In mostcases, extensive damage of the pancreas is requiredbefore diabetes occurs and the exocrine function of thepancreas is decreased in these patients. Cirrhosis incystic fibrosis may contribute to insulin resistance anddiabetes.

Diabetes mellitus is commonest endocrine disorder that affects more than 100 million people worldwide (6% population). It is caused by deficiency or ineffective production of insulin by pancreas which results in increase or decrease in concentrations of glucose in the blood. It is found to damage many of body systems particularly blood vessels, eyes, kidney, heart and nerves. The presence of diabetes mellitus shows increased risk of many complications such as cardiovascular diseases, peripheral vascular diseases, stroke, neuropathy, renal failure, retinopathy, blindness, amputations etc. Drugs are used primarily to save life and alleviate symptoms. Secondary aims are to prevent long-term diabetic complications and, by eliminating various risk factors, to increase longevity. Insulin replacement therapy is the mainstay for patients with type 1 diabetes mellitus while diet and lifestyle modifications are considered the cornerstone for the treatment and management of type 2 diabetes mellitus. The main disadvantage of currently available drugs is that they have to be given throughout the life and produce side effects . Medicinal plants and their bioactive constituents can be used for treatment of diabetes mellitus throughout the world especially in countries where access to the conventional anti diabetes mellitus agents is inadequate.

1.2 Symptoms

Diabetes symptoms vary depending on how much your blood sugar is elevated. Some people, especially those with prediabetes or type 2 diabetes, may sometimes not experience symptoms. In type 1 diabetes, symptoms tend to come on quickly and be more severe.

Some of the signs and symptoms of type 1 diabetes and type 2 diabetes are:

- Increased thirst
- Frequent urination
- Extreme hunger
- Unexplained weight loss
- Presence of ketones in the urine
- Fatigue
- Irritability
- Blurred vision
- Slow-healing sores
- Frequent infections, such as gums or skin infections and vaginal infections

1.3 CAUSES OF DIABETES

Causes of type 1 diabetes

The exact cause of type 1 diabetes is unknown. What is known is that your immune system which normally fights harmful bacteria or viruses attacks and destroys your insulin-producing cells in the pancreas. This leaves you with little or no insulin. Instead of being transported into your cells, sugar builds up in your bloodstream. Type-1 is thought to be caused by a combination of genetic susceptibility and environmental factors, though exactly what those factors are is still unclear. Weight is not believed to be a factor in type-1 diabetes.

Causes of prediabetes and type 2 diabetes

In prediabetes which can lead to type-2 diabetes and in type-2 diabetes, your cells become resistant to the action of insulin, and your pancreas is unable to make enough insulin to overcome this resistance. Instead of moving into your cells where it's needed for energy, sugar builds up in your bloodstream. Exactly why this happens is uncertain, although it's believed that genetic and

environmental factors play a role in the development of type-2 diabetes too. Being overweight is strongly linked to the development of type 2 diabetes, but not everyone with type-2 is overweight.

2. MATERIAL AND METHOD

2.1 Collection

Fresh leaves of *Cordia sinensis* were collected from area adjoining forests of Bhopal in the month of October, 2019.

2.2 Extraction of plant material

Dried powdered of leaves of *Cordia sinensis* has been extracted with 80% ethanol using maceration process for 24 hrs, filtered and dried using vaccum evaporator at 40^0C.

2.3 Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

2.4 Phytochemical Screening

Cordia sinensis leaves extract was subjected to the precursory phytochemical analysis following standard methods by Khandelwal and Kokate. The extract was screened to identify the presence of various active principles of alkaloids, glycosides, phenols, flavonoids, Terpenoids, Saponins, Steroids.

2.5 Estimation of total Phenolic and flavonoid Content

2.5.1 Total Phenolic content estimation

Procedure: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol.10 mg of dried extracted dissolve in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenols. 2 ml of extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min for color development. The absorbance was measured at 765 nm using a spectrophotometer.

2.5.2 Total flavonoids content estimation

Procedure 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of extract dissolved in 10 ml methanol and filter. Three (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

2.6 *In -Vivo* Anti diabetic activity

2.6.1 Animals:-

Wistar rats (180–225 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C). Rats received standard rodent chow and water *ad libitum* (OECD, 2002). Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

2.6.2 Induction of Experimental Diabetes in Rats

After fasting, diabetes was induced by a single intraperitoneal injection of 120 mg/kg body weight of 'Alloxan monohydrate' in distilled water. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. These

animals were tested for diabetes after 15 days and animals with blood glucose (fasting) were selected for experimentation (Adediwura and Abo Kio, 2008; Pareek *et al.*, 2009).

2.6.3 Experimental Protocol

Animals were divided into five groups of 6 rats each (Pareek *et al.*, 2009).

Group I: Rats served as normal-control and received the vehicle (0.5 ml distilled water/day/rat)

Group II: Rats served as diabetic-control and received the vehicle (0.5 ml distilled water/day/rat)

Group III: Rats (diabetic) were administered of hydroalcoholic extract of *Cordia sinensis* (100 mg/kg p.o.) for 15 days

Group IV: Rats (diabetic) were administered of hydroalcoholic extract of *Cordia sinensis* (200 mg/kg p.o.) for 15 days

Group V: Rats (diabetic) were administered Glibenclamide (600µg/kg p.o.) for 15 days.

3. RESULTS AND DISCUSSION

3.1 Determination of Percentage Yield

Table 3.1: % Yield of hydroalcoholic extract of *Cordia sinensis*

S. No.	Extract	% Yield
1.	Hydro alcoholic	7.5

3.2 Result of Phytochemical screening of extract

Table 3.2 Phytochemical screening of hydroalcoholic extract of leaves extract of *Cordia sinensis*

S. No.	Test	Extract
		Hydro alcoholic extract
1	**Alkaloids** a) Wagner's Test: b) Hager's Test:	+ve
2	**Carbohydrates** a) Fehling's Test:	+ve
3	**Glycosides:** a) Legal's Test:	+ve
4	**Saponins** a) Froth Test:	+ve

5	**Phenols** a) Ferric Chloride Test:	+ve
6	**Flavonoids** a) Alkaline Reagent Test: b) Lead acetate Test:	+ve -ve
7	**Proteins** a) Xanthoproteic Test:	-ve
8	**Diterpenes** a) Copper acetate Test:	-ve

+ ve – **Present, - ve – Absent**

3.3 Results of Estimation of Total Phenolic Contents and Total flavonoid content

Table -3.3: Results of Estimation of total phenol and total flavonoids content *Cordia sinensis* extract

S. No.	Extract	Total Phenol Content	Total Flavonoids Content
1.	Hydroalcoholic	0.895	0.747

3.4 Results of *in vivo* anti diabetic activity

Table 3.4: Effect of hydroalcoholic extract of *Cordia sinensis* treatment on blood glucose (mg/dl) in normal and diabetic rats

Values are expressed as mean±S.E.M (n = 6).Values are statistically significant at $^{\#}$p<0.001 vs. normal group; *P < 0.001,

Group	Treatment	Blood glucose (mg/dl)		
		Days 0	Days 8	Days 15
I	Normal	91.70± 5.50	96.25 ± 5.50	101.25± 5.50
II	Diabetic Control	270.50 ± 10.35	284.50± 9.35$^{\#}$	290.50± 8.35$^{\#}$
III	Diabetic + hydroalcoholic extract of *Cordia sinensis* (100 mg/kg)	242.50 ± 4.10	157.50± 4.70***	121.50 ± 3.25***
IV	Diabetic + hydroalcoholic extract of *Cordia sinensis* (200 mg/kg)	253.85 ± 3.00	148.35 ± 4.10***	119.150 ± 4.63***
V	Diabetic + Glibenclamide (600µg/kg)	250.00 ± 4.90	140.00± 4.90***	110.00 ± 4.90***

**P < 0.01vs. diabetic control group (Two-way ANOVA test).

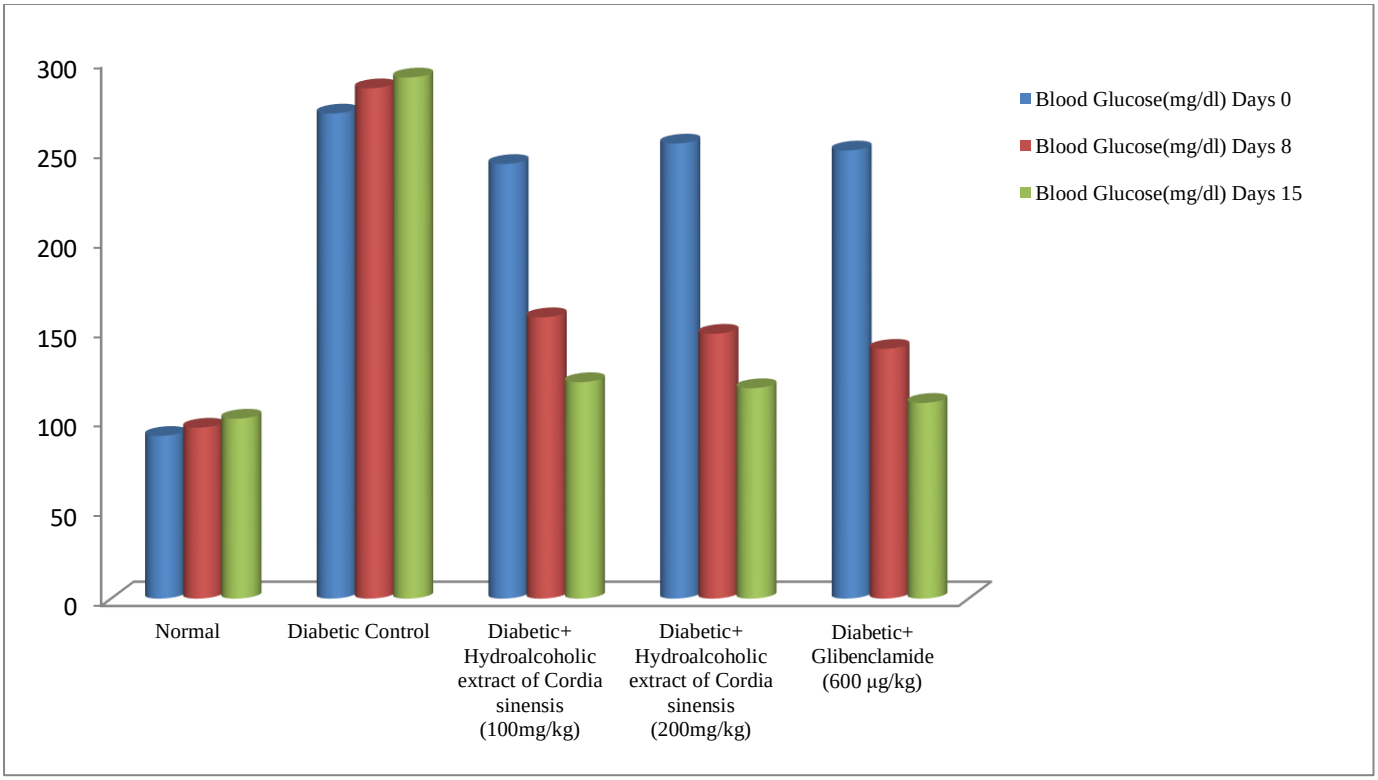

Figure 3.1: Effect of hydroalcoholic extract of *Cordia sinensis* treatment on blood glucose (mg/dl) in normal and diabetic rats

Table 3.5: Effect of hydroalcoholic extract of *Cordia sinensis* treatment on biochemical parameters in normal and diabetic rats

Values are expressed as mean±S.E.M (n = 6).Values are statistically significant at $^{\#}p<0.001$ vs. normal group; *$P < 0.001$, **$P < 0.01$vs. diabetic control group (One-way ANOVA followed by Tukey's post hoc test).

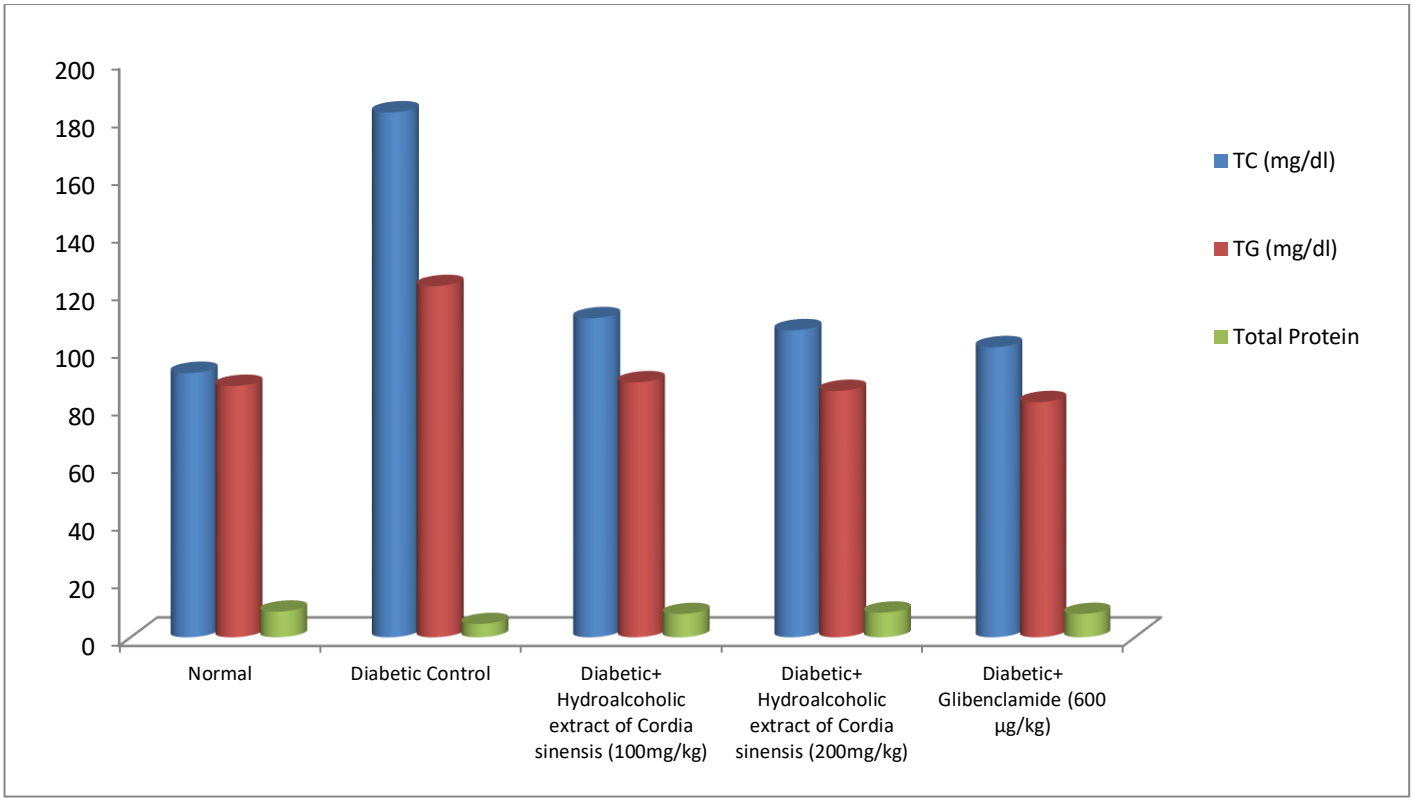

Figure 3.2: Effect of hydroalcoholic extract of *Cordia sinensis* treatment on total cholesterol, triglyceride, and Total protein in normal and diabetic rats

Table 3.6: Effects of hydroalcoholic extract of *Cordia sinensis* on body weight

Group	Treatment	Initial weight (g)	Final weight (g)
I	Normal	180.00 ± 8.00	205.10 ± 9.00
II	Diabetic Control	185.00 ± 8.00	175.00 ±9.00
III	Diabetic + hydroalcoholic extract of *Cordia sinensis* (100 mg/kg)	190.00 ± 8.00	202.50 ± 8.00
IV	Diabetic + hydroalcoholic extract of *Cordia sinensis* (200 mg/kg)	195.00 ± 8.00	208.25 ± 9.00
V	Diabetic + Glibenclamide (600µg/kg)	200.00 ± 8.00	223.95 ± 9.00

Values are expressed as mean ± SD of six samples from each group. (Two-way ANOVA test).

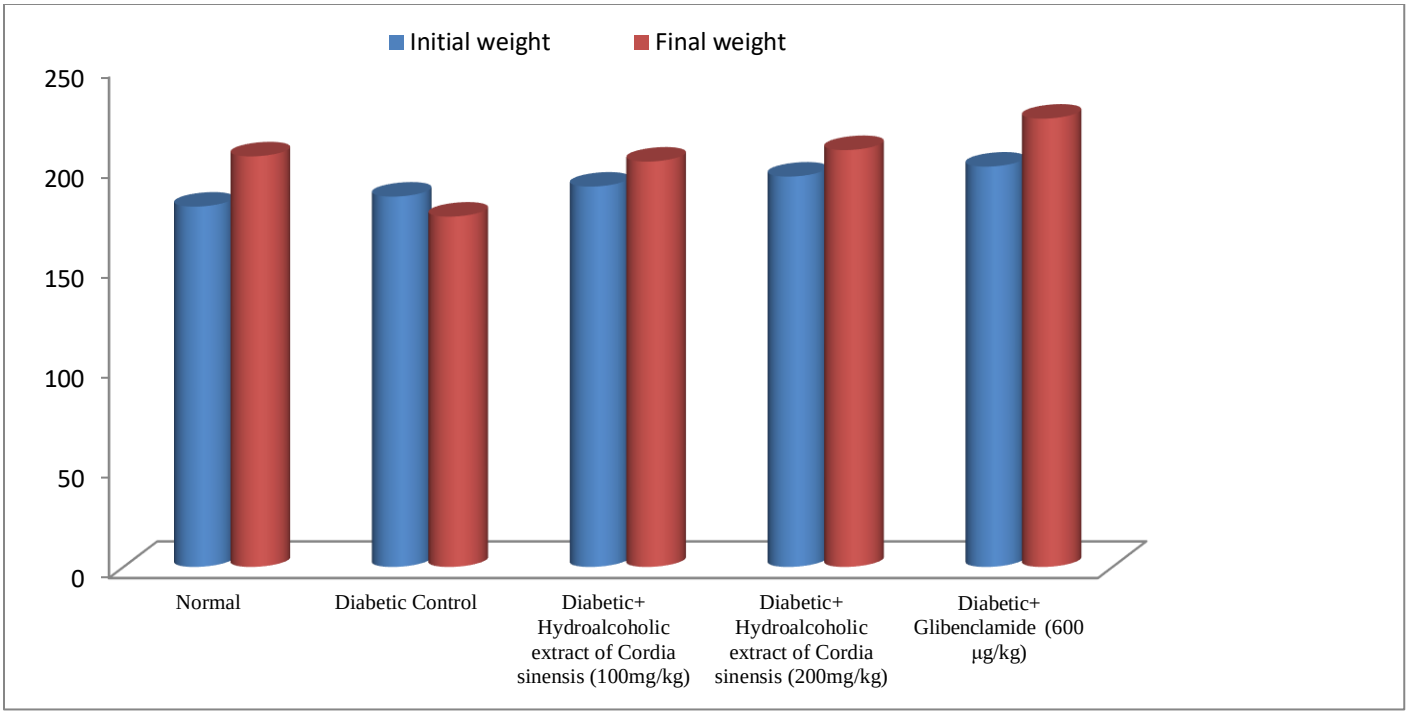

Figure 3.3: Effects of hydroalcoholic extract of *Cordia sinensis* on body weight

CONCLUSION

Phytochemical screening of *Cordia sinensis* leaves reveals the presences of Alkaloids, Saponins, Glycosides, Carbohydrates, Flavonoids and Phenols. The total phenolic content was found 0.895 mg/100mg of dry weight of extract, expressed as gallic acid equivalents and the total flavonoid content was found 0.747mg/100mg, expressed as Quercetin equivalents. Hydroalcoholic extract of *Cordia sinensis* exhibited significant anti-hyperglycemic activities in alloxan-induced hyperglycemic rats without significant change in body weight; they can also improve the condition of Diabetic mellitus as indicated by parameters like body weight & lipid profile. The renewal of cells in diabetes has been studied in several animal models. The total cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet ß cells following destruction by alloxan may be the primary cause of the recovery of alloxan-injected guinea pigs from the effects of the drug. Hydroalcoholic extract of *Cordia sinensis* has been shown to act by cell regeneration. In our studies, the damage of pancreas in alloxan-treated diabetic control rats and regeneration of cells by glibenclamide was observed. It is found that of hydroalcoholic extract of *Cordia sinensis* at high dose (200 mg/kg) is more effective than whole plant extract at low dose (100 mg/kg) after 15 days of treatment. Hence the above discussion revels that of hydroalcoholic extract of *Cordia sinensis* at high dose (200 mg/kg) is more effective and shows similar curative effect as standard that is, glibenclamide (600 µg/kg). This could be due to the possibility that some -cells are still surviving to act upon by hydroalcoholic leaves extract of *Cordia sinensis* to exert its insulin releasing effect. Hydroalcoholic leaves extract of *Cordia sinensis* at high dose (200 mg/kg) exhibited significant antihyperglycemic activity in alloxan-induced diabetic rats. These extracts also showed improvement in parameters like body weight and lipid profile as well as

regeneration of cells of pancreas and so might be of value in diabetes treatment.

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