EVALUATION OF ANTIDIABETIC POTENTIAL OF *CORCHORUS DEPRESSUS* - A CHOLISTANI PLANT IN CONTROLLING DIABETES MELLITUS THROUGH IN-VIVO STUDIES.

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Abstract-Diabetes mellitus is a metabolic syndrome characterized by hyperglycemia and abnormal metabolism of lipids and proteins. From the "Stone Ages", plants and their derived products have been considered valuable sources for humankind's survival and well-being. Pakistan is endowed with rich medicinal flora and most of it remains unexplored. Local herbal practitioners use diverse varieties of plants without doing any systematic standardization. This research comprises standardization of the antidiabetic plant "*Corchorus depressus"* with special reference to biological fingerprinting through in-vivo studies using animal models. Methanolic extracts of *Corchorus depressus* vide 100 mg/kg body weight, and its fractions (aqueous, ethyl acetate, and petroleum ether vide 30mg/kg and 100mg/kg were administered orally to "Albino Sprague-Dawley" male rats. Results were monitored at 0, 1st, 7th, and 14th day after single-dose administration. Streptozotocin (40mg/kg/2ml) in citrate buffer of pH 4.5 was used for induction of diabetes mellitus. The parameters studied were fasting blood glucose, serum biochemical testing, and histopathology of animals. Significance of different treatments was assessed through statistical analysis of data. Crude extracts of *C. depressus* significantly lowered the fasting blood sugar compared to positive control (462±35) during the study period. Effects of Cd. Cr 100 mg/kg (116±26.5***) was comparable to metformin (Met 10 mg/kg: 133±4.55***). The aqueous and ethyl acetate fraction of *C. depressus* doses vide 30mg/kg (138.6±22.6***) and 100 mg/kg (143.6±10.2***) lowered down the fasting blood glucose highly significantly as compared to positive control throughout the study period. The effects of Cd. E.A 30mg/kg (155±15.8***) and Cd. E.A 100 mg/kg (122±3.5***) was more comparable to Met 10 mg/kg. While petroleum ether fraction Cd. P.E 30mg/kg (286±9.1), and Cd. P.E 100mg/kg (208.6±9.2**) did not show much comparable results. However, an alleviated level of lipid (Cholesterol, Triglycerides), hepatic panel (ALT, ALP, Bilirubin), and renal panel (Creatinine, Urea) was noted in streptozotocin-applied diabetic rats. Administration of Cd crude and all its fractions significantly (p<0.05) renovated all biochemical parameters. Moreover, histopathology of organs like kidneys, pancreas, and liver have revealed the selected plant has potential to lower down the noxious effects of streptozotocin. The also study justifies the use of *Corchorus depressus* for the control and management of diabetes mellitus.

Index Term- Diabetes mellitus, Streptozotocin, Petroleum ether, Ethyl acetate, Aqueous, *Corchorus depressus*.

I. INTRODUCTION

The history of Diabetes in Subcontinent dates back to 6th century AD. It was recognized as due to excess of sugar by some of the Hindu physicians. Diabetes is a set of illnesses characterized by elevated blood sugar (glucose) levels. The pancreas's limited ability to produce insulin causes type-I diabetes. A person with type 1 diabetes has an entire lack of insulin, and between 5 and 10 percent of persons have this condition (Malviya et al., 2010). Diabetes often refers as Diabetes mellitus and is commonly known as "hyperglycemia" mainly occurred due to deficiency of Insulin secreted by β-cells. Insulin is required to consume glucose from digested food as an energy source, as a result of chronic hyperglycemia micro vascular and macro vascular complication may occur leading to blindness, kidney problems, heart diseases etc (Vana et al., 2019). Insulin controls hepatic glucose output by increasing glycogen synthesis and inhabiting glycogenolysis. Increased rate of hepatic glucose production results in "Overt hyperglycemia" commonly fasting hyperglycemia. So, "Diabetes is actually the chronic elevation of blood glucose level", which is accompanied by polyurea, severe hunger, acute thirst, weight loss and even leads to death in case of absence of proper treatment.

Physicians employed traditional herbs (medicinal plants) in the past to treat conditions including cancer, diabetes, and heart disease (Elavarasi et al., 2013, Verma et al., 2018). Polyphenolic substances were found in the plant species that had anti-diabetic benefits. (Sanlier and Gencer, 2020). Non-pharmacological therapies for diabetes have recently attracted a lot of attention. The usage of herbal medications is the most often used as alternative medicines for blood sugar management (Kumar et al., 2018). Worldwide, diabetic patients increased from 108 M (1980) to 422 M (2014), and meanwhile diabetes caused nearly 1.6 million deaths. Additionally, it has been expected that the number of diabetic patients will increase up to 300 million by 2025. WHO reported that diabetes would be the 7th prominent disease that cause death in 2030 (Skalli et al., 2019). Likewise, certain food additives known as non-nutritive sweeteners (Ass) are also recommended to patients that sweeten the food with no or few calories (Raveendran et al., 2018).

There is a need to create anti-diabetic medications that are safe, effective, and have fewer adverse effects (Saxena and Vikram, 2004; Ullah et al., 2022). Studies have confirmed the efficacy and safety of traditional herbs for cure of high blood sugar (Salimifar et al., 2013). Research studies have confirmed that many plants have ability to control and cure diabetes. Plants have many phytochemicals including flavonoids, terpenoids, alkaloids, glycosides, or carotenoids (Malviya et al., 2010). This study was designed for evaluation of the antidiabetic activity of *Corchorus depressus* (*Tiliaceae*).

C. depressus, often regarded as a good binder of sand in the desert, is a perennial shrub, prostrate *i.e.*, lying closely against the ground surface. Fruit is encapsulated, 8-15mm long, cylindrical, and curving upwards from the underside of the branches. Seeds are spatted, brown, and minuscule in size (Mathur and Sundaramoorthy, 2009). *C. depressus* is wide spread throughout the world and it occurs most commonly in Tropical Africa, Afghanistan, Arabian Peninsula, India, and Pakistan (Mathur and Sundaramoorthy, 2008).

Various flavonoids such as apigenin, luteolin, depressonol-A, and depressonol-B are present in *Corchorus depressus* (Zahid et al., 2002), quercetin and kaempferol (Harsh and Nag, 1988). The whole plant of *C. depressus* contains depressoside-A and B, and β-sitosterol (Nag and Harsh, 1982; Ahmad et al., 1998). Many *Corchorus* species are having important bioactive compounds such as cardiac glycosides, stropanthidin, terpenoid-corosin, flavone glycoside, urasolic acid, vitamin C, b-carotene, and mucilage, *etc.* (Sen, 2002). Evaluations over a period of time have shown use of *C. depprusses* as anti-malarial (Simonsen et al., 2001),

antifungal, antibacterial (Harsh et al., 1983; Harsh and Nag, 1988), analgesic, and antipyretic activities (Vohora et al., 1981; Khan et al., 2006). Its decoction is used as an emollient as good cooling agent as well as tonic (Khan et al., 2013) to cure spermatorrhea and premature ejaculation (Qureshi and Bhatti, 2008). *C. depressus* is well-known to cure gonorrhea, diabetes, and treachery troubles, and to improve sexual vigor among the local inhabitants of Rajasthan and Cholistan (Choudhary et al., 2017). The chloroform fraction of *C. depressus* has potency as aphrodisiac activity in normal male albino rats without any gastric ulceration and bad effects up to 400 mg/kg body weight (Kataria et al., 2013). Aerial and root parts of methanolic and dichloromethane fractions of aerial and root portions of *C. depressus* possess antioxidant activity, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and α-glucosidase inhibitory activities (Afzal et al., 2017).

High amounts of flavonoids, especially quercetin and kaempferol are present in *C. depressus*. These flavonoids have potential to control myeloperoxidase release or its activity, which leads to lower down the production of active oxygen species which leads to anticancer and anti-mutagenic activities (Hart et al., 1990; Kumari et al., 2019).

II. Material and Methods

Plant Collection

Plants of *C. depressus* were collected from different areas of Cholistan desert, Bahawalpur (Punjab-Pakistan), the latitude of Cholistan desert is 28^0 and longitude is 71^0, respectively. Plant Taxonomist Mr. Muhammad Waris of Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur identified plant specimen. The plant specimen (Voucher No. 3471/CIDS/IUB) was deposited in the herbarium of CIDS for future reference. Eight kilograms of fresh plant material was chopped and shade dried. Afterward, plant material was grinded into a fine powder with the help of a grinder (Eapmic-RY102).

Preparation of Crude Extract and Fractions

The fine powder of *C. depressus* was macerated in methanol for 8 days then filtered and the residue was again dipped in methanol, dipped in a container containing 10 liters of methanol. The container was then sealed completely for maceration. On day 9, the mixture was well shaken, and filtered with the help of filter paper (Whatsman filter paper of Grade 3). This procedure was repeated three times and about 6 liters of the filtrate (crude extract) was obtained finally. The filtrate was filled in a round bottom flask of 1L and was brought to a rotary evaporator for evaporation of methanol, this filtrate was evaporated in a vacuum on low pressure (Hayat et al., 2014). The crude methanolic extract was taken in an empty China dish (237g), and air dried. For the collection of crude extract, above mentioned procedure was repeated thrice. The final weight of the crude gummy extract of *C. depressus* was 403g along with the weight of empty China dish. In the second phase of the experiment, the fractions of crude extract were made with different chemicals using a simple solvent-solvent extraction method, and was performed in a series of non-polar to polar chemicals i.e., petroleum ether, ethyl-acetate. Finally, added distilled water to raise the volume. The fractions were obtained using a separating funnel (Rashid et al., 2020).

IN-VIVO STUDIES (BIOLOGICAL SCREENING)

Use Experimental Animals

Wistar albino rats of male sex having body weight of 180-300g were selected for this study. All the rats were placed in the experimental zone 01 of the Pharmacology Research Laboratory at the Faculty of Pharmacy, the IUB Bahawalpur. Three animals trialed in the experiment were kept in wooden cages (Dim. 47×34×18 cm^3) at room temperature 25 ± 1 °C and 50% ± 5% humidity. The acclimatization is necessary to reduce the animal

stress. The rules, criteria, and plan of action were permitted by honorable committee of animal supervision of the department of Pharmacy, The Islamia University of Bahawalpur.

Induction of Diabetes mellitus

After the acclimatization of rats, diabetes was induced in them. Animals are properly labeled and put on approximately 8 hours fasting following provision of water rat animals as normal. The weight of animals was measured with the help of weighing balance (Analytical balance of Fisher scientific). All the animals were divided randomly into control and experimental groups. Control group was just injected with citrate buffer. The intoxicated group was injected with streptozotocin (STZ) for pharmacological induction of diabetes mellitus in animals. Blood glucose level was measured at 0 day using Gluco-meter of Accu- check Performa, (Roche, Germany). A glucose solution (20%) was given to rats after the STZ induction to avoid the hypoglycemic condition in rats (Qamar et al., 2011) followed by provision of 5% glucose solution to rats for next 24 hours. STZ was prepared just before the induction and used it within 5 min of dissolution, and was injected intraperitoneally (IP) at 40 mg/kg/2ml in the animals of all experimental groups. In normal control group animals, an equal volume of citrate buffer (pH 4.5) was injected, intraperitoneally (Qamar et al., 2011).

Experimental Model

After induction of STZ, the blood glucose level at 3rd day of first treatment assured the presence of diabetes mellitus (DM). Then, animals were retained for 10 days to stabilize themselves. Rats were divided into various groups on 14th day. The fasting blood glucose levels of rats were measured, and treatment was initiated on the same day by considering it "the day one" of the study (Yashwant Kumar et al., 2011). Further assessment of fasting blood glucose level was carried out on 0-day, 7th day and 14th days of the study. The treatment design followed is as under:

Group I: Normal Control (Citrate Buffer 2 ml/kg + Distilled water)
Group II: Positive Control (Streptozotocin 40mg/kg/2ml + Distilled water)
Group III: Standard Control (Streptozotocin 40mg/kg/2ml+ Metformin 500mg/kg)
Group IV: Cd. Cr 100 mg/kg (Streptozotocin 40mg/kg/2ml+ Cd. Cr 100 mg/kg)
Group V: Cd. Aq 30 mg/kg (Streptozotocin 40mg/kg/2ml+ Cd. Aq 30 mg/kg)
Group VI: Cd. Aq 100 mg/kg (Streptozotocin 40mg/kg/2ml+ Cd. Aq 100 mg/kg)
Group VII: Cd. PE 30 mg/kg (Streptozotocin 40mg/kg/2ml+ Cd. P.E 100 mg/kg)
Group VIII: Cd. PE 100 mg/kg (Streptozotocin 40mg/kg/2ml+ Cd. P.E 30 mg/kg)
Group IX: Cd. EA 30 mg/kg (Streptozotocin 40mg/kg/2ml+ Cd. EA 30 mg/kg)
Group X: Cd. EA 100 mg/kg (Streptozotocin 40mg/kg/2ml+ Cd. EA 100 mg/kg)

Blood Collection and sample processing

Blood glucose levels of rats were measured by the use of a glucometer (Accu- check Performa, Roche-Germany). The test strips of gluco-meter were able to measure blood glucose levels in 10 mg/dl - 600 mg/dl range. By cervical dislocation, the rats were killed, and blood samples were taken to separate the serum from the plasma for further examination. Blood samples were centrifuged for 10 minutes at 4000 rpm at 4^{0}C. Then, the collected serum and plasma were stowed at -20 ^{0}C for further biochemical and antioxidant analysis. Different parts of rats i.e., pancreas, kidneys and liver were dissected out, preserved in 10% neutral buffered formalin (NBF), and used for histo-pathological evaluation (Mahmood et al., 2020).

Biochemical Testing

Biochemical analysis of serum obtained from treated rats was performed for lipid profile, liver and function of the rats following the method of Balamash et al., (2018).

Lipid Profile

Lipid profiling of obtained serum was determined by using commercially available kits (Gesan Productions, Italy), and a chemical analyzer (Selectra, Netherlands). Total cholesterol (mg/dL) and triglycerides (mg/dL) were the main focus of lipid profile.

Liver Function Tests

Liver function tests was carried out through alanine transaminase (ALT; U/L), alkaline phosphates (ALP; U/L), and bilirubin (mg/dL). For this purpose, commercially available kits (Gesan Productions, Italy), and a chemical analyzer (Selectra, Netherlands) was used.

Renal Function Tests

Creatinine and serum urea level was estimated in serum samples by using kits (Gesan Productions, Italy), and a chemistry analyzer (Selectra, Netherlands).

Paraffin embedding and histochemical techniques

For light microscopic studies, the organs like pancreas, kidneys, and liver were fixed in 10% neutral phosphate-buffered formalin (PBS, pH 6.8-7.4) solutions, and further processed by the routine tissue paraffin technique as described by (Suvarna et al., 2013). The microscopic examination was executed using 40x objectives.

Statistical Analysis

Results were presented as means with standard errors. Analysis of variance (ANOVA) was used to statistically analyses the data. In order to determine statistical significance at P(<0.05), t-test was used. Graph-pad Prism was used to compile for statistically analyses the data.

III. RESULTS

Hypoglycemic Effect of *C. depressus*

Results revealed streptozotocin group showed highest blood glucose level during 15 days of treatment. Metformin (standard drug) lower down the blood glucose level highly significantly about 71% decrease as compared to streptozotocin. While crude extract of *C. depressus* (Cd.cr.100mg/kg) also lowered down the blood glucose level of treated animals and showed 74% decrease in blood glucose level during in-vivo study as compared to diabetic control group (Table 1). This shows that *C. depressus* contains some hypoglycemic agents which are responsible for controlling diabetes mellitus. Further fractions of *C. depressus*; petroleum ether fraction (Cd. P.E.), Ethyl acetate fraction (Cd. E.A.) and Aqueous fraction (Cd. Aq.) were used in two concentrations *viz.* 30mg/kg and 100mg/kg. Aqueous 30mg/kg and 100mglkg gave 71% and 68% decrease in blood glucose level respectively as compared to streptozotocin. While ethyl acetate fractions dose 30 mg/kg showed highly significant results (P<0.001) decrease (62%) of blood glucose and ethyl acetate fraction of 100 mg/kg showed 73% decrease in blood glucose as compared to streptozotocin Petroleum ether fraction 30mg/kg was not significant and gave 38% decrease in blood glucose, however in petroleum ether fraction of 100mg/kg more significant (*P<0.01*) 54% decrease in glucose level was observed (Table 1). Hence, *C. depressus* crude extract (Cd.cr 100mg/kg), aqueous fraction (Cd. Aq 30mg/kg) and ethyl acetate (Cd. EA 100mg/kg) has more potency to control diabetes mellitus in comparison with Metformin (standard drug).

Table: 1. Effect of *C. depressus* crude extract (Cd.cr 100mg/kg), aqueous fraction (Cd. Aq 30mg/kg and 100mg/kg), ethyl acetate fraction (Cd. E.A. 30mg/kg and 100mg/kg), and petroleum ether fraction of *Corchorus depressus* (Cd. P.E. 30mg/kg and 100mg/kg) on fasting blood glucose (mg/dL) of STZ induced diabetic rats.

S. No.	Treatment group	Days							
		Induction Days					Treatment days		
		0	3	7	10	14	0	7	14
1	Normal control	74.6±4.1	72±10	80.6±3.7	78±1.52	74.3±6.74	74.3±6.7	80.3±3.84	77±2.08
2	Diabetic control	91.5±9	366±78	368±72	375±72.4	365±78	365±78	374±71	462±35
3	Standard (Met)	109±12.6	314±43	306±39	331±61	367±52.8	367±52.8	176±16.2*	133±4.55***
4	Cd. Cr. 100	82.6±6.5	412±85	429±89	409.6±78	393±60	393±60	148±4.4*	116±26.5***
5	Cd. Aq.30mg/kg	87.6±6	261±12	261±21.4	232.6±9.9	261.6±12	261.6±12	136.3±29*	138.6±22.6***
6	Cd. Aq. 100mg/kg	90±3.7	402.3±90.8	359.6±104.5	354±95	352.6±89.2	352.6±89.2	145.3±2.6**	143.6±10.2***
7	Cd. E.A 30mg/kg	100±1.7	478±61	485±52	487±55	465±70	465±70	252±27**	155±15.8***
8	Cd. E.A. 100mg/kg	92±4	351±47	357.6±40.9	375.6±43	371±53	371±53	137±9.8**	122±3.5***
9	Cd. P.E. 30mg/kg	90±9.7	361.6±95	401.6±99	391±104	371±116.9	371±116.7	270.6±27	286±9.1
10	Cd. P.E.100mg/kg	110±8.7	341±17	376.6±56.8	400.6±42.4	408.6±27	408.6±27	228.6±19	208.6±9.2**

Restorative Effect of *C. depressus* on Liver Function:

Effect of metformin (MET) and plant extracts on hyperglycemic animals was assessed, and treatment showed significant responses. Mean values for serum alkaline phosphatase (ALP) level were significantly high in streptozotocin treated groups as compared to the control group. Metformin significantly reduces about 35% decrease in the mean serum ALP level. There was 62% decrease in ALP level in animals treated with *C. depressus* crude extract (Cd.cr 100mg/kg). Aqueous fraction (Cd. Aq. 100mg/kg) showed 42% decrease, ethyl acetate fraction (Cd. EA100 mg/kg) reduces about 61% of serum ALP and petroleum ether fraction of *Corchorus depressus* (Cd. P.E 100mg/kg) showed 22% increase in serum ALP. Hence, crude extract of *Corchorus depressus*, its aqueous and ethyl acetate fraction has more potency to drop mean serum ALP ratio in comparison with metformin, but petroleum ether fraction showed increase in ALP level.

There was a significant decrease in mean serum alkaline transaminase (ALT), except animals treated with aqueous fraction (Cd. Aq.). Metformin significantly reduces about 25% decrease in the mean serum ALT level. There was 45% decrease in ALT level in animals treated with *C. depressus* crude extract (Cd.cr 100mg/kg). Aqueous fraction (Cd. Aq. 100mg/kg) showed no decrease, ethye acetate fraction (Cd. EA100 mg/kg) reduces about 82% of serum ALT and petroleum ether fraction of *Corchorus depressus* (Cd. P.E 100mg/kg) showed 27% decrease in serum ALT. Hence, crude extract of *Corchorus depressus*, its aqueous and petroleum ether fraction showed no significant drop in mean serum ALT ratio in comparison with metformin, but ethyl acetate fraction showed good decrease in ALT level.

However, bilirubin was not much significantly decreased in all fractions of *C. depressus*, as compared to diabetic controlled group animals. Metformin showed about 57% decrease in the mean serum bilirubin level. There was 42% decrease in bilirubin level in animals treated with *C. depressus* crude extract (Cd.cr 100mg/kg).

Aqueous fraction (Cd. Aq. 100mg/kg) showed 71% decrease, ethyl acetate fraction (Cd. EA100 mg/kg) reduces about 28% of serum bilirubin and petroleum ether fraction of *Corchorus depressus* (Cd. P.E 100mg/kg) showed 28% decrease in serum bilirubin. Hence, crude extract of *Corchorus depressus*, its ethyl acetate fraction and petroleum ether did not respond well to drop mean serum bilirubin ratio in comparison with metformin, but aqueous fraction showed significant decrease in bilirubin level (Table 2).

Table: 2. Liver function markers, renal function markers, and lipid profile of animals treated with plant *C. depressus.*

S. No.	Treatment groups	Liver Function Markers			Renal Function Markers		Lipid Profile	
		ALP (U/L)	ALT (U/L)	Bilirubin (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Cholesterol	Triglycerides
1	Normal Control (NC)	216±0.5	35±0.5	0.5±0.05	36±0.5	0.5±0.05	66±0.5	82 ±0.5
2	Diabetic Control (DC)	712±1.2	94±0.5	0.7±0.05	81±0.5	0.7±0.03	69±0.5	46±0.5
3	Standard Control (STD)	459±0.5***	70±0.5***	0.3±0.05*	84±0.5	0.6±0.05	69±0.5	46±0.5
4	Crude Extract (Cr.100mg/kg)	269±0.5***	51±0.5***	0.4±0.05*	76±0.5	0.6±0.05	76±0.5	52±0.5***
5	Aqueous Fraction (Aq.100mg/kg)	410±0.5***	94±0.5	0.2±0.05	48±0.5***	0.7±0.05	48±0.5***	40±0.5
6	Ethyl Acetate (E.A.100mg/kg)	276±0.5***	16±0.5***	0.5±0.05	23±0.5***	0.04±0.05***	23±0.5***	39±0.5***
7	Petroleum Ether (P.E.100mg/kg)	870±0.5***	68±0.5***	0.5±0.05	7±0.5***	0.3±0.1***	7±0.5***	12±0.5***

Values are expressed as Mean ± SEM and n = 5. Student t-test was used. P- values are considered as $P < 0.05$ = significant (*), $P < 0.01$ = more significant (**) and $P < 0.001$ = highly significant (***). All extract treated groups and standard controlled were compared to positive control and positive control was compared to normal control.

Restorative Effect of *C. depressus* on Renal Function Markers

Results have shown a pretentious effect of treatment on different renal function parameters in treated rat animals. Mean urea level was significantly high in streptozotocin treated groups, compared to control group. Metformin not tended to reduce the mean urea level and about 3% increase in the mean serum urea level was observed. There was 6% decrease in urea level in animals treated with *C. depressus* crude extract (Cd.cr 100mg/kg). Aqueous fraction (Cd. Aq. 100mg/kg) showed 40% decrease, ethyl acetate fraction (Cd. EA100 mg/kg) reduces about 65% of serum urea and petroleum ether fraction of *Corchorus depressus* (Cd.P.E 100mg/kg) showed 91% decrease in serum urea. Hence, crude extract of *Corchorus depressus*, its aqueous and ethyl acetate fraction did not respond well to drop mean serum urea ratio in comparison with metformin, but petroleum ether fraction showed significant decrease in urea level (Table 2).

Likewise, mean creatinine level was also significantly high in streptozotocin treated groups. Metformin reduces 14% of the mean creatinine level. There was 14% decrease in creatinine level in animals treated with *C. depressus* crude extract (Cd.cr 100mg/kg). Aqueous fraction (Cd. Aq. 100mg/kg) showed no decrease, ethyl acetate fraction (Cd. EA100 mg/kg) reduces about 94% of serum creatinine and petroleum ether fraction of *Corchoruss depressus* (Cd. P.E 100mg/kg) showed 57% decrease in serum creatinine. Hence, crude extract of *Corchorus depressus*, its aqueous fraction did not respond well to drop mean serum urea ratio in comparison with

metformin, but petroleum ether fraction showed significant decrease in urea level and ethyl acetate fraction drop creatinine level significantly (Table 2).

Effect of C. depressus on Lipid Profile

Effect of streptozotocin treatment showed a high level of total cholesterol level in streptozotocin treated groups as compared to control group. Metformin not tended to reduce the mean serum total cholesterol level, there was 0% decrease. Statistical analysis also revealed 10% decrease in mean serum total cholesterol level in group treated with crude extract of *C. depressus* (Cd. Cr.) and aqueous fraction (Cd. Aq.) group showed 30% decrease, ethyl acetate fraction (Cd. E.A.) at 100mg/kg gave 66% decrease, and petroleum ether fraction of *C. depressus* (Cd. P.E) lowered 89% of the serum cholesterol (Table 2).

Mean serum total triglycerides level was significantly high in streptozotocin treated groups. No reduction in mean serum total triglycerides level was observed due to metformin. A 13% decrease in mean serum total triglycerides level was noted in animal group treated with crude extract of *C. depressus* (Cd. Cr.) and aqueous fraction of this plant (Cd. Aq.). Moreover, ethyl acetate fraction (Cd. E.A.) gave 15 % decrease and petroleum ether fraction (Cd. P.E) at 100mg/kg of *C. depressus* have significantly ($P \leq 0.01$) decreased the mean serum total triglycerides up to 73 percent (Table 2).

HISTOPATHOLOGICAL STUDIES OF ANIMALS TREATED WITH *C. DEPRESSUS*

Histopathology of Liver of Rats Treated with *C. depressus*

Hepatic parenchyma of the control group in photomicrograph (plate 1) exhibited normal sinusoids, normal liver portal area morphology, and normal liver cells (a). Photomicrographs of the hepatic cells in the diabetes control group (STZ) revealed congestion and necrosis (b). The hepatic triad and cells in the Metformin-treated group, however, appeared almost normal (c). The hepatocytes in the group of rats treated with *C. depressus* crude extract (100 mg/kg) seemed nearly normal on the photomicrograph (d). Hepatic region showed a light to moderate amount of congestion and a few necrotic cells was present in the photomicrograph of the *C. depressus* aqueous fraction 100mg/kg (Cd. Aq) further this treated group revealed a minor degree of congestion and edema in hepatic cells, indicating a partial ameliorative effect (e). A photomicrograph of the *C. depressus* group treated with 100 mg/kg of ethyl acetate (Cd. E.A) showed that the treatment had a good ameliorative effect as evidenced by the fusion of the portal triad and a minor necrosis in the hepatocytes (f). In the photomicrograph of the *C. depressus* petroleum ether 100 mg/kg treated group (Cd. P.E.), congestion and fatty vacuoles in hepatocytes, as well as the fusion of the portal triad, showed a poor ameliorative effect (g).

Histopathology of Kidneys of *Corchorus depressus* Treated Group:

Plate 2 (a-g) shows treatment effect on kidneys of rat animals. Kidneys of control group in photomicrograph showed normal glomeruli and normal renal tubules (a). Kidneys of diabetic control showed congestion of glomeruli and peritubular capillaries (b), and kidneys of the metformin treated group showed almost normal appearance of kidneys and normal cellularity (c). Kidneys of *Corchorus depressus* crude extract 100mg/kg (Cd. Cr) treated group showed glomeruli that exhibited modest necrotic alterations(d). Kidneys of rat group treated with *C. depressus* aqueous fraction 100mg/kg (Cd. Aq) showed less congestion and less vacuolization(e), while kidneys of rat group treated with *C. depressus* ethyl acetate 100mg/kg (Cd. E.A) showed no inflammatory changes and no necrosis at all (f). Likewise, *C. depressus* petroleum ether 100mg/kg (Cd. P.E) treated group showed reduced size of glomeruli and shedding of glomeruli (g).

Histopathology of Pancreas of *Corchorus depressus* Treated Group

Plate 3 (a-g) shows treatment effect on pancreas histology. Pancreas of the control group in the photomicrograph displayed normal pancreatic cells and completely active islets of Langerhans in the pancreatic parenchyma (a). The pancreas of a diabetic control group had relatively tiny islets of Langerhans, destroyed cells, and cellular contents lost (b). The metformin-treated group's pancreas demonstrated nearly normal appearance and healthy cell nuclei (c). Likewise, *C. depressus* crude extract 100mg/kg (Cd. Cr) treated group showed pancreas with minor necrotic alterations in the pancreatic cells (d). The pancreas of *C. depressus* aqueous fraction (100mg/kg of Cd. Aq) had fewer Langerhans islets and pancreatic cell swelling (e), while the pancreas of rat animals treated with 100 mg/kg of *C. depressus* ethyl acetate fraction (Cd. E.A) had nearly normal pancreatic parenchyma and active islet of Langerhans (f). *C. depressus* petroleum ether 100 mg/kg (Cd. P.E.) showed reduced size of islets of Langerhans in pancreas of animals (g).

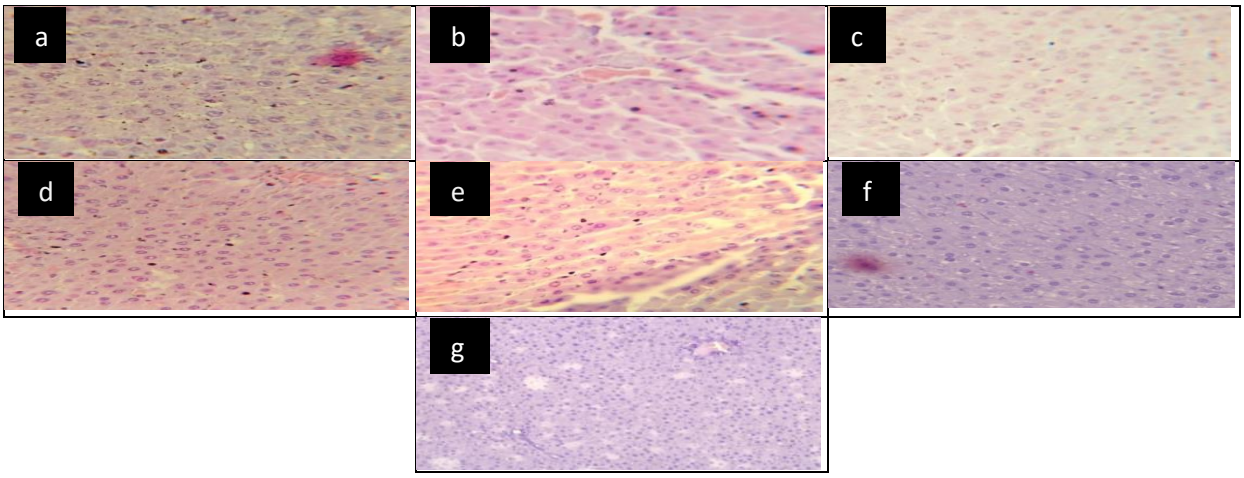

Plate 1: Photomicrographs of Liver sections of control, Diabetic control, Standard control, Cd. Cr 100mg/kg treated group, Cd.Aq.100mg/kg, Cd. E.A 100mg/kg and Cd. P.E. 100mg/kg treated groups of rats (H&E staining 40X).

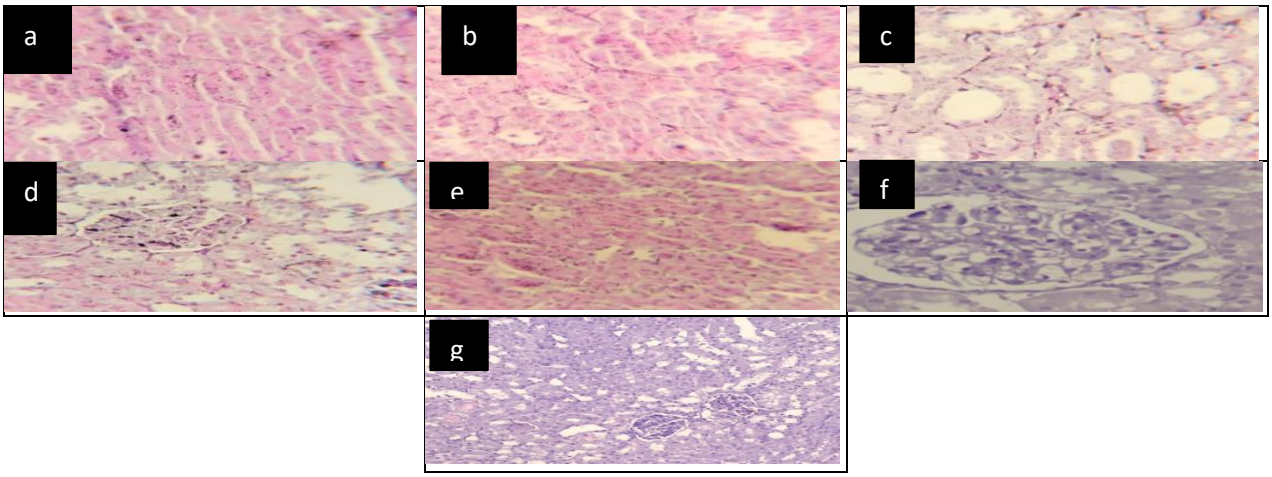

Plate 2: Photomicrographs of Kidneys sections of control, Diabetic control, Standard control, Cd. Cr 100mg/kg treated group, Cd.Aq.100mg/kg, Cd. E.A 100mg/kg and Cd. P.E. 100mg/kg treated groups of rats (H&E staining 40X).

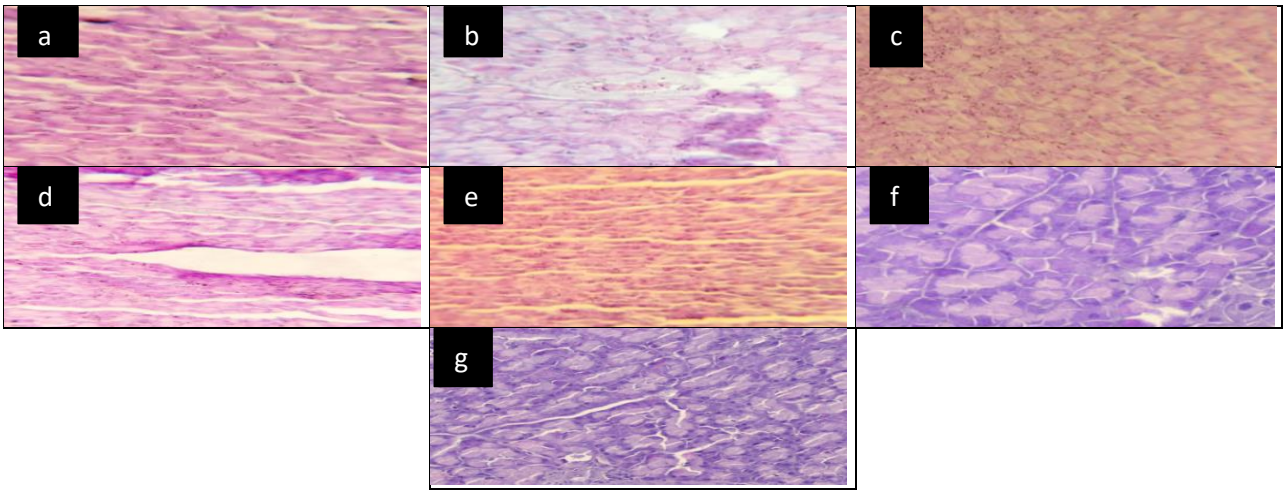

Plate 3: Photomicrographs of pancreas sections of control, Diabetic control, Standard control, Cd. Cr 100mg/kg treated group, Cd.Aq.100mg/kg, Cd. E.A 100mg/kg and Cd. P.E. 100mg/kg treated groups of rats (H&E staining 40X).

IV. Discussion

Corchorus depressus is a perennial shrub with prostrate spread pattern i.e. lying closely against the ground surface. *C. depressus* is distributed in almost all parts of the world including Pakistan (Mathur and Sundaramoorthy, 2009). Flavonoids such as apigenin, luteolin, depressonol- A, depressonol -B, quercetin, and kaempferol are abundant in this plant (Zahid et al., 2002). Important bioactive substances found in *Corchorus species* include urasolic acid, cardiac glycosides, stropanthidin, terpenoid-corosin, flavone glycoside, and mucilage contents (Chopra et al., 1986; Sen, 2002; Alqahtani et al., 2022). *C. depressus* whole plant extract (hexane & Chloroform soluble) exhibited strong antipyretic activity in rabbits that were injected with yeast injections hypodermically with no apparent toxic or detrimental effect up to the oral dose of 1.6 g/kg (Khan et al., 2006).

Blood glucose level (BGL) was monitored at various intervals like day 3, 7, 10 and day 14. Hyperglycemic activity or diabetes was confirmed in the rats after 72 hours of induction with streptozotocin (STZ) and the animals showing fasting blood glucose level ≥ 200-600 mg/dl were observed. All the rats with high blood glucose level were used for screening of anti-diabetic effects of crude extracts and extracts of various fractions of *C. depressus* (petroleum ether fraction, ethyl-acetate fraction and aqueous fraction). The results have shown that STZ significantly ($P \leq 0.01$) raised fasting blood glucose after diabetes induction. Treatment of diabetic rats with *C. depressus* crude extract and its different fractions; aqueous fraction (Aq), petroleum ether fraction (P.E) and ethyl acetate fraction (Cd) produced dose dependent decrease in fasting blood glucose *viz.* 30mg/kg and 100mg/kg. In present study, 100mg/kg dose was found to be more potent on 14^{th} day of treatment. Results reported by Ezeigbo et al., (2016) has supported the findings of present study that use of different doses of ethanolic and aqueous extracts of *M. oleifera* leaves in diabetic rats significantly decreased blood glucose level at 14^{th} day of treatment. The present study is also supported by findings of Subhasree et al., (2015) that discovered in streptozotocin (STZ)-induced diabetic rats the anti-hyperglycemic potential of a polyherbal formulation (PHF) made of hydroalcoholic extracts from four different plants like *Lagerstroemia parviflora, Salacia oblonga, S. roxbhurgii,* and *Garcinia*

indica. These results demonstrate that using a polyherbal formulation for 28 days caused a considerable reduction in blood glucose levels in diabetic rats.

By significantly decreasing cholesterol and triglyceride levels after treatment of diabetic rats with selected plants, the current study demonstrated the anti-hyperlipidemic actions of these plants. Petroleum ether These findings were validated by a second study in which diabetic rats' administration of the polyherbal medication "Diasulin" ethanolic extract considerably decreased their blood glucose levels, serum lipid profiles, and lipid peroxidation. (Saravanan and Pari, 2005).

The serum levels of liver function markers i.e. ALT, ALP, and bilirubin significantly increased in streptozotocin induced diabetic rats during this study metformin the standard drug worked well to control liver function marker. Furthermore, crude extract and ethyl acetate fraction showed much better results in lowering liver function markers. The integrity and degree of liver damage can be evaluated after exposure to any chemical or pharmacological substance, such as streptozotocin, utilizing the serum or plasma level of specific liver function enzymes (Lee et al., 2014 and Sadiq et al., 2020). In another study, rats with diabetes induced by streptozotocin exhibited noticeably higher serum levels of aminotransferases (Mathur et al., 2016). These results are supported by the fact that the use of ethanolic leaf extracts of *Cajanus cajan* and *Moringa oleifera* significantly reduced blood ALT, ALP, and bilirubin levels in alloxan-induced diabetic rats (Aja et al., 2015).

Likewise, renal parameters were found altered in diabetic induced rats significantly. Serum levels of urea and creatinine were elevated in streptozotocin-induced diabetic rats than in the control group. These features may extend towards damaged kidney functions such as glomerular and tubular hypertrophy, tubulointerstitial fibrosis or increased albumin-urea (Giacco and Brownlee, 2010) due to diabetes. In the present investigation, application of selected plant extract has significantly reversed serum creatinine and urea levels. These findings are consistent with a prior study in which diabetic groups of rats used an alcoholic extract of *Salvia hydrangea*, which reduced serum levels of urea nitrogen and creatinine (Zarei et al., 2015).

Plate 1 showed liver of normal group, hepatic parenchyma showed normal appearance of hepatic cell, normal sinusoids and normal appearance of hepatic portal area while diabetic control group (Streptozotocin STZ) showed congestion and necrosis of hepatic cells, metformin treated group (MET) showed almost normal appearance of cells and normal appearance of hepatic triad**.** Moreover, animal group treated with crude extract (100 mg/kg) indicated almost normal appearance of hepatocytes and mild to moderate congestion and few necrotic cells in hepatic area. Aqueous fraction 100mg/kg (Aq) treated group showed a mild degree of congestion and edema in hepatic cells indicating partial ameliorative effect. Ethyl aetate 100 mg/kg treated group (E.A) indicated good ameliorative effect as indicated by slight necrosis in hepatocytes and fusion of portal triad. Petroleum Ether 100 mg/kg treated group (P.E.) indicated poor ameliorative effect as indicated by congestion and fatty vacuole in hepatocytes and fusion of portal triad. Hepatic cell congestion was visible upon histopathological evaluation of stained liver slices from rats with diabetes brought on by streptozotocin. Hepatocytes with a normal appearance and mild to moderate congestion were dramatically improved in diabetic rats treated with the plant extract's highest dose (100 mg/kg).

The findings of earlier study by El Latif et al., 2014 has indicated the pancreas and liver of rats treated with *M. oleifera* and rats with alloxan-induced diabetes were histopathologically examined. The findings showed that alloxan killed pancreatic cells, causing atrophy. Together with substantial liver degeneration and portal and central venous congestion, diabetic rats' livers also displayed these symptoms. However, administration of 250

mg/kg of *M. oleifera* leaf extract significantly reversed the degenerative alterations in diabetic rats, with only a modest increase in congestion in the islets and hepatocyte of Langerhans.

Similarly, Francis and Sudha (2016) have reported destruction of islets of Langerhans due to congestion of pancreas in STZ induced diabetic rats. Streptozotocin-induced diabetic rats revealed destroyed islets of Langerhans with prominent congestion, and infiltration in the affected pancreas. Extracts of different plants i.e., *Phyllanthus emblica, Syzygium aromaticum, Curcuma Longa, Piper longum, Tinospora cordifolia, Phyllanthus amarus,* and *Moringa oleifera* were used in a polyherbal formulation to treat hyperglycemic rats, and the results indicated normal acinar cells. Cytoplasmic vacuolation and lymphocytic infiltration were not present.

V. CONCLUSION:

Corchorus depressus is a wild cholistani-plant and is safe to use to treat diabetic patients. Results have revealed that its crude extract and aqueous as well as ethyl acetate fraction have a great potency to control and cure diabetes in streptozotocin induced diabetic rats at dose of 100 mg/kg body weight as compared to 30 mg/kg with less significant results. *C. depressus* is rich in flavonoids, the main chemical constituent in controlling diabetes. Hence, the chemical composition of *C. depressus* extract can be used to replace antidiabetic drugs. More research on the extra acts of *C. depressus* is needed in order to isolate and identify the active compound (s) responsible for its anti-diabetic potential and standardize the therapeutic markers on a scientific basis with the expectation that they will be beneficial for mankind with fewer side effects. In vivo research and clinical trials should be conducted on the newly discovered chemicals from these plants in order to confirm that they are natural medicinal agents. Based on the findings of present research, *C. depressus* is recommended as an antidiabetic plant source.

Conflict of Interest

The authors declare no conflict of interest.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

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