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Ameliorative Potentials Of Ethanolic Leaves Extracts Of *Cassia occidentalis* And *Pithecellobium dulce* On Diabetes Associated Metabolic Alterations In Streptozotocin-Induced Diabetic Rats: A Comparative Study

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Abstract:
A comparative analysis was performed in this study to detect the ameliorative potentials of Ethanolic leaves extracts (EL) of *Cassia occidentalis* and *Pithecellobium dulce* medicinal plants by oral administration of 200mg/kg bw and 400mg/kg bw individually and in combination on streptozotocin-induced diabetic rats. In diabetic rats, the treatment of EL extracts of both plant species (1:1) in combination of 400 mg/kg bw doses and time following significantly reduced blood glucose level (101.67± 9.38 mg/dl) and body weight (201.67± 9.38 g) to normal. Individual plant EL extracts also demonstrated a successful response in diabetic rats at doses of 200 and 400 mg/kg bw in restoring insulin and hepatic glycogen levels to near-normal levels. In both normal and diabetic rats, total cholesterol, triglycerides, AST, ALT, carbohydrates metabolic enzymes (Hexokinase, Glucose-6-Phosphatase, Fructose 1, 6 Bisphospahtes), and antioxidative enzymes (SOD, CAT, and GPx) were measured. In STZ-diabetic rats, oral administration of EL extracts from both plants in combination significantly and dose-dependently normalized the above serum and liver parameters ($p<0.05$). Histopathological analysis revealed a protective and restorative effect.

Key words: Antidiabetic activity, Streptozotocin, Insulin, Ethanolic Extracts, Pancreas.

1] Introduction:

Diabetes mellitus (DM) is a highly variable, adlerian endocrine disorder characterized by chronic hyperglycemia that persists due to defects in insulin secretion, action, or both [1]. DM is marked by the appearance of hypercholesterolemia, hypertriglyceridemia, and low serum high-density lipoprotein cholesterol (HDL-C) in diabetics compared to normal individuals [2-3]. Chronic hyperglycemia causes microvascular impediments that affect the nerves, kidneys, and eyes, as well as increasing the risk of cardiovascular disease [4].

According to the International Diabetes Federation (IDF), the global burden of diabetes was 366 million in 2011 and is expected to rise to 552 million by 2030. Despite newer and more successful treatment strategies, new diagnostic devices, strict glycemic targets, better treatment guidelines, and increased disease awareness, baseline glycosylated haemoglobin remains relatively high in diabetic subjects [5]. In India, this disorder is at an alarmingly high level when compared to the majority of developed countries. By 2025, India is expected to have the most people with Diabetes Mellitus in the world [6]. Despite advances in disorder understanding and management, the disease's mortality and morbidity are increasing [7].

Anti-diabetic medications currently available, such as sulfonylureas, biguanides, β- glucosidase inhibitors, thiazolidinediones, and insulin, are frequently associated with undesirable side effects or a decrease in response after prolonged use [1]. With today's diabetes drugs, achieving better glycemic control with minimal side effects and ease of access remains a challenge. Nowadays, the use of medicinal plants is increasing because treating diabetes with phytotherapy is affordable, easily accessible, less expensive, very effective, and has fewer side effects [8]. Traditional medicine is used for primary health care by approximately 70–90% of the population in developed and developing countries because it is relatively inexpensive and has fewer side effects [9]. Natural products are important in the discovery of new therapeutic agents and have received a lot of attention as sources of bioactive substances such as antioxidants, hypoglycemic and hypolipidemic agents [10]. Because herbal preparations are regarded as a primary source of modern medicine, these facts have encouraged the

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expansion of the frontiers of scientific evaluation of the hypoglycemic properties of various plant species. [11-12].
Cassia occidentalis and *Pithecellobium dulce* are members of the same Fabaceae family and have been shown to have a variety of therapeutic effects [13-14]. Taking traditional claims and reported activities into account, *C. occidentalis* and *P. dulce* have been studied for their Diabetes Associated Metabolic Alterations in Streptozotocin –Induced Diabetic Rats. In light of this, the current study was undertaken to determine the synergistic anti-diabetic activity of the combination of these plants and compare its potential in streptozotocin induced diabetic rats [15].

2] Materials and Methods:
Plants collection and Authentication: Dr. A. Amurtha Valli, Associate Professor, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India, authenticated *C. occidentalis* and *P. dulce* plant materials collected from local village areas of Nellore district, Andhra Pradesh state. Before being shade dried for 10 days, the plant leaves were thoroughly washed, first with tap water and then with distilled water, to remove any debris or dust particles. The dried leaves were ground to a fine powder in a sterile mixer grinder for 5 minutes before being stored at room temperature in airtight plastic containers.

Preparation of the Leaf Extract: 500 g of previously prepared powder was mixed with 1500 ml of the solvent ethanol and left undisturbed overnight at 10°C. The solution was then allowed to stand in a boiling water bath for 1-2 hours, with occasional shaking, before being left undisturbed for 24 hours. The extract was concentrated using a rotaevaporator under vacuum below 40°C after being filtered through sterile Whatman No.1 filter paper [16-17]. The dried extract was then exposed to UV light for 24 hours before being tested for sterility on nutrient agar plates and stored in labeled sterile bottles in a freezer at 4°C until further use [18].

Preliminary Phytochemical Screening: To analyze the secondary metabolites (phytochemical) constituents present in the various solvent extracts, various standard protocols were used [19-21].

Animal Collection: Adult healthy albino Wistar rats (175 to 250 g) of both sexes were obtained from the Animal House, Ratnam Institute of Pharmacy, Nellore, Andhra Pradesh, India, and housed in the Department of Pharmacology under standard husbandry conditions (30°C ± 5; 60-70% relative humidity and 12 hrs day night cycle). They were fed a standard rat pellet diet and were given unlimited access to water. All animal procedures were approved by the Institutional Animal Ethical Committee, and the experiment was carried out in accordance with the CPCSEA Guidelines.

Acute Oral Toxicity Study of the EL Extracts: An acute oral toxicity study of the EL extracts of *C. occidentalis* and *P. dulce* was carried out in accordance with the guidelines of the Organization for Economic Cooperation and Development (OECD) [22]. Prior to oral dosing, the rats were fasted overnight and given only water. The extract was then administered orally at various dose levels of 100, 200, 500, 1000, 1500, and 2000 mg/kg of body weight. The rats were monitored continuously for 24 hours for behavioral and adverse changes, and then for lethality.

Diabetes induction and experimental design: The experimental rats were fasted overnight, and Streptozotocin (STZ) was weighed individually for each rat before being injected intraperitoneally with STZ (50 mg/kg bw) in freshly prepared 0.1M of cold citrate buffer (pH 4.5) [23-24]. The rats were given free access to food and water after 30 minutes of injection. After 48 hours of STZ administration, the blood glucose level was estimated. Animals with fasting blood glucose levels greater than 200-350 gm/dl were considered diabetic and were chosen for the next activity. 48 hours after STZ injection, treatment with EL extracts *C. occidentalis* and *P. dulce* was initiated. The chosen animals were divided into nine groups of six each.

Group I: A normal control group that did not receive STZ injections received a daily dose of 0.3 ml of 2.5 percent Tween 80 (vehicle) p.o.
Group II: Diabetic control with a single intravenous dose of 50 mg/kg bw STZ.
Group III: Diabetic control with a daily dose of 5 mg/kg bw Glibenclamide in 2.5 percent Tween 80 p.o.
Group IV: Diabetic control with a daily dose of 200 mg/kg bw ELCO in 2.5 percent Tween 80 p.o.
Group V: Diabetic control with 400 mg/kg bw ELCO in 2.5 percent Tween 80 p.o. daily dose.
Group VI: Diabetic control with a daily dose of 200 mg/kg bw ELPD in a 2.5 percent Tween 80 p.o.

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solution.
Group VII: Diabetic control with 400 mg/kg bw ELPD daily dose in 2.5 percent Tween 80 p.o.
Group VIII: Diabetic control with a daily dose of 200 mg/kg bw ELCO & ELPD (1:1) in 2.5 percent Tween 80 p.o.
Group IX: Diabetic control with 400 mg/kg bw ELCO & ELPD (1:1) in 2.5 percent Tween 80 p.o.

Biochemical Assays: A commercially available glucose oxidase kit was used to analyze fasting blood glucose levels (Span Diagnostics Ltd, Surat, India). The Elisa kit from Stat Diagnostics, Mumbai, was used to measure plasma insulin levels. The liver was immediately dissected, washed in ice-cold saline to remove blood, and homogenized in 0.1M Tris –HCl buffer, pH 7.4. The supernatant was tested for carbohydrate metabolic enzyme activity using Trinder (1969) [25] (Hexokinase), King (1965) [26] (Glucose-6-Phosphatase), and Gancedo and Gancedo (1971) [27] (Fructose-1,6 Bisphospahtes). Morales *et al*. (1975) [28] Total cholesterol (Parekh and Jung, 1970) [29], and Total Triglycerides (Foster and Dunn, 1973) [29]. Enzymes from the liver (AST, ALT) (Reitmann and Frankel, 1957) [30]. Antioxidant enzymes (SOD) (Kakkar *et al*., 1984) [31], CAT (Sinha, 1972) [32], Rotruck *et al*., 1973) [33] (GPx).

Histopathological studies: At the end of the experiments, the animals are sacrificed under sedation (Isoflurane). The liver and pancreas of one animal from each group were then excised, washed with normal saline, and stored in 10% formalin solution for histopathological analysis. The tissue was washed, dehydrated in alcohol, cleared in xylene, and paraffin blocks were created. A semi-automated rotary microtome was used to cut serial sections of 5 m thickness. The sections were deparaffinized with xylene and rehydrated in descending grades of ethanol before being stained with haematoxylin and eosin and examined under a microscope.

Statistical analysis: The results are expressed as the mean SEM of the number of experiments. To determine the significance of changes, the data was subjected to analysis of variance (one way ANOVA), followed by Dunnett's test.

3] Results and Discussion:
As a first step, a preliminary phytochemical analysis was performed on both EL leaves extracts of *C. occidentalis* and *P. dulce* medicinal plants, both of which are members of the Fabaceae family. The presence of alkaloids, flavonoids, anthraquinones, tannins, proteins, and terpinoids was discovered **(Table 1)**. Acute oral toxicity studies on both plant species revealed no behavioral changes in the first 24 hours at doses less than 2000mg/kg bw. Normal rats treated with ET extract in the range of 100-1500 mg/kg bw for both plants had a 100% survival rate.
Diabetes is a metabolic disorder that affects a large proportion of the world's population. Depending on the type and severity of diabetes, treatment is based on the variable use and combination of diet, anti-diabetic oral agents (metformin, sulphonylureas, acarbose, and thiazolidinediones), and insulin or its analogues. Traditional diabetes treatments have many flaws, such as unfavorable side effects and a high rate of secondary complications. In general, there is little scientific knowledge on the specific mechanisms of action in the treatment of diabetes, but most plants have been found to contain active ingredients such as flavonoids, alkaloids, glycosides, terpenoids, and so on, which have anti-diabetic properties [35, 36]. The presence of flavonoids, alkaloids, glycosides, polyphenols, tannins, saponins, phytosterols, and triterpenes in the EL leaves extract was revealed by phytochemical analysis **(Table 1)**. Diabetes prevention and treatment rely heavily on phytocompound-based strategies [37]. Polyphenolic compounds, such as flavonoids, help to increase plasma antioxidant capacity, lower oxidative stress markers, and lower total and LDL cholesterol [38].
A growing body of evidence suggests that different dietary phytophenols may influence carbohydrate metabolism at multiple levels [39]. Phytochemicals are compounds with various biological properties that enable plants to cope with environmental challenges such as radiation and toxins [40]. They are bioactive compounds (secondary metabolites) found in plants that work in conjunction with nutrients and dietary fibres to provide disease protection [41]. The presence of biologically active ingredients in individual plant EL extracts and in combination doses could explain the observed pharmacological effects.

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Table 1: Comparative Phytoconstituents analysis in EL extracts of *C occidentalis* (CO) and *P. dulce* (PD).

S.N0	Phytochemicals	Test's Applied	ELCO	ELPD
1	**Alkaloids**	a) Mayer's Test b) Dragendroff's Test c) Wagner's Test	++++	+++
2	**Saponins**	Foam Test	–	–
3	**Flavanoids**	a) Ferric Chloride Test b) Shinoida's Test	++++	++
4	**Anthraquinones**	Borntrager's Test	++	++
5	**Tannins**	a) Ferric Chloride Test b) Lead Acetate Test	++	++
6	**Sterols**	a) Salkowski's Test b) Libermann-Burchards Test	–	+
7	**Sugars**	a) Barfoed's Test b) Molish's Test	++	++
8	**Cardiac Glycosides**	a) Borntrager's Test b) Balget's Test	–	–
9	**Lipds and Oils**	Saponification Test	+	+
10	**Coumarins**	Sodium Hydroxide Test	++	–
11	**Proteins**	a) Biuret's Test b) Ninhydrin Test	+	+
12	**Terpinoids**	Salkowski Test	++	+++

In rats, diabetes was induced by a single intraperitoneal injection of STZ. Animals with severe hyperglycemia (glucose >200 mg/dl) were compared to rats with normal blood sugar levels after 48 hours. Group I rats had normal blood glucose levels and were classified as Normal, whereas Groups II- IX were classified as diabetic. For 21 days, ELCO 200 mg/kg bw and 400 mg/kg bw, ELPD 200 mg/kg and 400 mg/kg, and a combined plant extract (1:1) of ELCO and ELPD at doses of 200 mg/kg and 400 mg/kg bw were administered orally. **Table 2** compares the effect of EL extracts of ELCO, ELPD, and combined plant extract (1:1) of ELCO and ELPD at doses of 200 mg/kg and 400 mg/kg on non- diabetic rats' mean fasting blood sugar to diabetic rats' mean fasting blood sugar. Blood glucose levels were significantly lower after treatment with EL plant extract. At a dose of 400 mg/kg, the combined plant extract of ELCO and ELPD showed significant and promising signs of anti-diabetic activity against STZ-induced diabetic rats, and the effect was comparable to that of the standard drug glibenclamide (5 mg/kg).

Table 2: Effect of STZ, Standard and EL extracts on blood glucose level in different groups of normal and diabetic rats.

Groups	Pre-treatment	Post-treatment		
	Day 0	**Day 7**	**Day 14**	**Day 21**
Normal control	87.64 ± 5.42	89.45 ± 7.62	90.76 ± 4.43	91.07 ± 8.45
Diabetic control	289.5 ± 6.123	324.5 ± 10.47	380.5 ± 14.11	408.5 ± 15.68
Glibenclamide 5mg/kg bw	268.17 ± 15.33	212.17 ± 10.41	162.17 ±12.47	96.17 ± 8.75
200 mg/kg bw ELCO	224.33 ± 14.23	220.65 ± 9.65	180.33 ± 10.84	133.17 ± 11.12*
400 mg/kg bwELCO	254.67 ± 17.84	236.33 ± 18.68	147.17 ± 15.41	102.67 ± 40.07**
200 mg/kg bwELPD	265.33 ± 9.38	244.67 ± 18.12	194.67 ± 14.56	148.83 ± 13.74*
400 mg/kg bwELPD	268.45± 9.36	235.54± 9.18	170.23± 8.38	109 .32± 10.38*
200 mg/kg bw ELCO&ELPD (1:1)	251.12 ± 8.34	224.14± 6.38	187.89 ± 9.18	150.45± 9.38
400 mg/kg bw ELCO&ELPD(1:1)	255.05 ± 9.68	215.12± 9.38	164.13 ± 7.38	101.67 ± 9.38**

Each value represents the mean ± SEM, (n=6) *P < 0.05, Vs diabetic control (ANOVA followed by Dunnett'stest). ELCO = Ethanolic leaves extract of *C.occidentalis* and ELPD = Ethanolic leaves extract of *P. dulce.*

Fig: 1 depicts the effect of various plant extracts on the mean body weights of non-diabetic rats versus diabetic rats. Diabetes induced by STZ is associated with a characteristic loss of body weight, which is most likely due to muscle wasting and protein loss in the tissues. The extracts ELCO, ELPD, and combined plant extract (1:1) ELCO and ELPD were given orally to different groups of diabetes animals for 21 days at doses

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of 200 mg/kg and 400 mg/kg. It was discovered that there is a significant increase in body weights after 21 days. The body weight of the STZ-induced untreated diabetic group decreased significantly in our study. DM was caused by STZ injection, most likely due to destruction of the β-cells of the pancreatic islets of Langerhans [42] Hyperglycemia in DM is caused by an excess of glucose production and a decrease in tissue utilization [13]. In comparison to diabetic control rats and rats treated with glibenclamide, oral administration of EL at a dose of 400 mg/kg for 21 days resulted in an improvement in body weight.

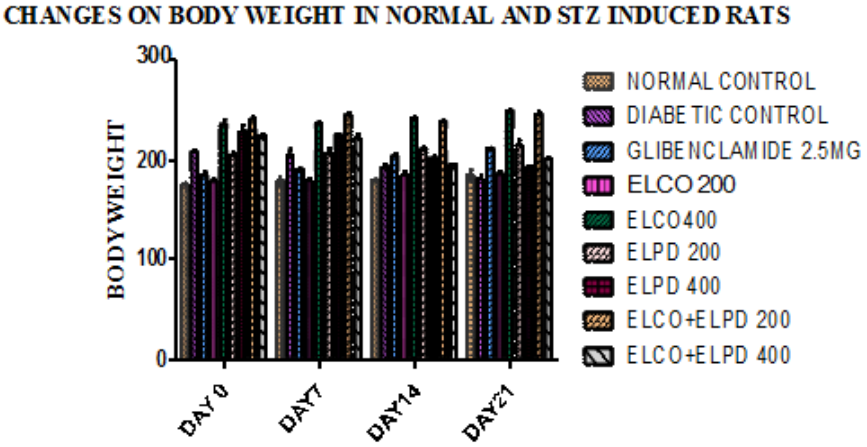

Fig 1: Effect of STZ, Standard and ET extracts on body weight (g) in different groups of normal and diabetic rats.

When plasma insulin and liver glycogen levels were studied, treated diabetic rats' levels were significantly restored to near normal levels **(Table 3).** In normal rats, plasma insulin and liver glycogen levels were 33.06± 0.514 (U/ml) and 50.06± 0.215 (mg/d wet tissue), respectively. The combined dose of 400 mg/kg bw of EL extract in (1:1) was 34.36 ±1.28 (U/ml) and 48.56± 1.24 (mg/d wet tissue). Because of STZ's ability to selectively target and destroy insulin-producing pancreatic β- islet-cells, STZ-induced hyperglycemia is a widely used experimental model. STZ (50 mg/kg) intraperitoneal administration partially damages insulin secreting pancreatic β-cells by breaking the DNA strand, resulting in increased blood glucose levels and decreased endogenous insulin release. The increased plasma insulin levels in EL-treated STZ-diabetic rats could be attributed to the protection of function β-cells from further deterioration. Increased insulin levels may aid in the improvement of glycemic control in STZ-diabetic rats. This demonstrated the presence of active anti-diabetic active principles in both the combination and individual doses of the plant's leaf.

Table 3: Effect of STZ, Standard and ET extracts on plasma insulin and Liver glycogen in different groups of normal and diabetic rats.

Groups	Parameters	
	Plasma Insulin (µU/ml)	Liver Glycogen (mg/d wet tissue)
Normal control	33.06 ± 0.514	50.06 ± 0.215
Diabetic control	14. 02± 0.506	21.64 ± 2.14
Glibenclamide (5mg/kgbw) p.o	30.18 ± 0.381*	45.82 ± 2.17*
200 mg/kgbw ELCO p.o	25.66 ± 0.42*	33.24 ± 3.56*
400mg/kgbw ELCO p.o	29.50 ± 0.92*	41.70 ± 1.12*
200 mg/kgbw ELPD p.o	27.81 ± 0.14*	43.11 ± 3.18**
400 mg/kgbw ELPD,p.o	29.54 ± 0.471*	47.18 ± 3.14*
200 mg/kgbw ELCO & ELPD (1:1) p.o	30.52 ± 0.24**	45.92 ± 2.22*
400 mg/kg ELCO & ELPD (1:1) p.o	34.36 ± 1.28*	48.56 ± 1.24**

Each value represents the mean ± SEM, (n=6) *P < 0.05, Vs diabetic control (ANOVA followed by Dunnett'stest). ELCO = Ethanolic leaves extract of *C. occidentalis* and ELPD = Ethanolic leaves extract of *P. dulce*.

The effect of ELCO and ELPD extracts on biochemical parameters such as total cholesterol, triglycerides, and liver enzymes SGOT and SGPT was also investigated and is depicted in **Fig: 2**. Diabetes is a metabolic disorder that causes changes in biochemical parameters. Triglyceride levels in

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diabetic rats were found to be elevated up to 113.60 mg/dl. This observation clearly demonstrated that changes in biochemical and liver function were also altered in diabetic rats. When ELCO, ELPD, and combined plant extract (1:1) of 200 mg/kg and 400 mg/kg treated rats were compared to diabetic control rats, there was a significant decrease in total cholesterol, triglyceride, SGOT, and SGPT. Hypertriglyceridemia and hypercholesterolemia are the most commonly observed lipid alterations in diabetes [43]. The presence of too many fatty acids in the bloodstream may aid in the hepatic conversion of fatty acids into phospholipids and cholesterol. These modifications are likely to result in secondary complications of diabetes, such as atherosclerosis and increased coronary heart disease. Our findings show that EL administration significantly reduced lipid profiles such as cholesterol and triglycerides.

It is important to measure the enzymes activities of aminotransferases SGOT and SGPT (AST and ALT). They were clinically and toxicologically significant because they were directly associated with the conversion of amino acids to Ketone acids are a type of acid. As a result, changes in their activities are indicative of tissue damage caused by toxicants or disease conditions. Transaminase levels in the liver are extremely high as a result of hepatocellular disorders [44]. The increase in plasma SGOT and SGPT activities suggested that diabetes may cause hepatic dysfunction due to liver necrosis [45]. When compared to the diabetic group, oral administration of EL extract and glibenclamide significantly reduces the activity of these enzymes. The return of diabetic rats' plasma AST, ALT levels to normal demonstrates that the EL extract has no negative effect on liver functions **(Fig: 2)**.

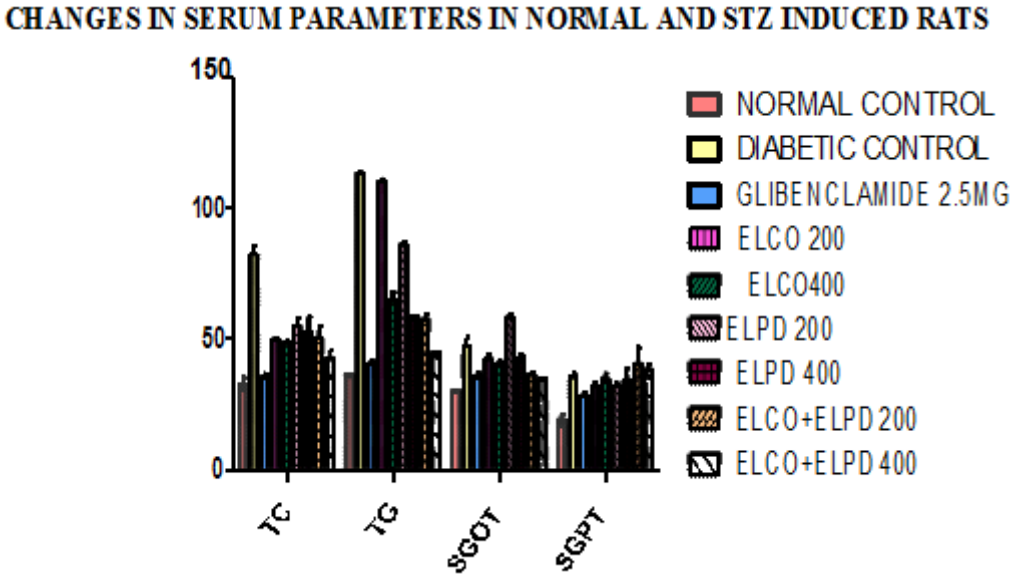

Fig 2: Effect of STZ, Standard and ET extracts on serum parameters in different groups of normal and diabetic rats.

Changes in the glycolytic enzyme (Hexokinase), gluconeogenesis enzyme (Glucose-6-phosphatase), and fructose 1, 6 bisphospatases were also monitored in the liver homogenate because this work is solely concerned with carbohydrate metabolism. A significant decrease in hepatic hexokinase levels was observed, with a concurrent increase in Glucose-6-phosphatase and Fructose 1, 6 bisphospatases levels **(Table 4)**. In STZ-induced diabetic rats, the alterations were nearly normalised after treatment with a combination dose of ET (400mg/kg bw). Hepatic glucose-6-phosphatase catalyses glucose production and is essential for maintaining blood glucose homeostasis. Increased glucose-6-phosphatase activity stimulates lipogenesis in diabetic rats by providing hydrogen to $NADP^+$, forming NADPH, and enhancing fat synthesis from carbohydrates [46], as well as increasing blood glucose levels [47]. The current study shows that hepatic glucose-6-phosphatase activity in diabetic rats was significantly higher than in normal rats, and that oral administration of EL extract and glibenclamide significantly reduced its activity and the amount of serum glucose released into the bloodstream. Fructose 1-6-phosphatase is a gluconeogenic pathway regulatory enzyme that catalyses the conversion of fructose-1, 6-bisphosphate to fructose-6-phosphate. In diabetics, activity of fructose 1-6-phosphatase increases, resulting in a decrease in glycolytic flux [48]. The oral administration of EL extract and glibenclamide reduces the activities of the gluconeogenic enzyme in diabetic rats **(Table 4)** by potentiating the effect of insulin release from β-islets of Langerhans cells, which may improve glucose utilization [49].

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Table 4: Effect of EL extract on carbohydrate metabolizing enzymes levels in control and STZ induced diabetic rats.

Groups	Hexokinase	Glucose-6-phosphatase	Fructose-1,6-bis phosphatase
Normal control	236.29 ± 1.54	1029. 8 ± 20.3	490.33 ± 18.22
Diabetic control	138.5 ± 2.37	1677.29 ± 45.20	756.9 ± 25.46[a]
Glibenclamide 2.5mg/kg bw	221. 34 ± 2.21[a]	1086.8 ± 35.29[a]	509.3 ± 12.67
200 mg/kg bw ELCO&ELPD (1:1)	217.67 ± 11.13[a]	1068.28 ± 53.20[b]	456.43 ± 21.31[b]
400 mg/kg bw ELCO&ELPD(1:1)	232. 50 ± 11.22[a]	999.82 ± 13.29[a]	473. 25 ± 1.28[b]

Each value is mean ± SD for 6 rats in each group. a: $p<0.05$ by comparison with normal rats. b: $p<0.05$ by comparison with STZ diabetic rats. ELCO = Ethanolic leaves extract of *C. occidentalis* and ELPD = Ethanolic leaves extract of *P. dulce*.

The observation on antioxidant enzymes also yielded positive results, as the levels of SOD, CAT, and GPx are drastically reduced due to the effect of STZ **(Table 5)**. When compared to the standard drug, diabetic rats responded well to the combination EL extracts of 200mg/kg and 400mg/kg bw. Oxidative stress is important in the development of hyperglycemia because it generates reactive oxygen species (ROS) that cause cellular injury and several deleterious effects on cellular physiology, all of which play a role in the development of secondary diabetes complications. Several studies have shown that STZ-treated β-cells produce oxygen free radicals and those antioxidant enzymes such as SOD and CAT are over expressed. Diabetes has been linked to decreased SOD and CAT activities in the liver. SOD is a critical defense enzyme that catalyses superoxide radical dismutation. CAT is a heme protein that catalyses hydrogen peroxide reduction and protects tissues from highly reactive hydroxyl radicals [50]. Low catalase activity has been linked to schizophrenia and atherosclerosis [51], and it is thought that long-term oxidative stress may lead to the development of Type II diabetes. In diabetic rats, oral administration of EL extract and glibenclamide significantly increases SOD, CAT, GPx levels **(Table 5)**. The EL extract's ability to restore the altered antioxidant enzymes and glutathione in STZ-induced diabetic rats suggests that it has free radical scavenging potential.

Table 5: Effect of EL extract on Antioxidative enzymes in control and STZ – diabetic rats.

Groups	SOD (units/min / mg protein)	CAT (n moles/ 100 g tissue)	GPx (µg of SSH consumed/ min/mg protein)
	LIVER		
Normal control	9.17±0.77	83.41±6.72	9.52±0.69
Diabetic control	4.12±0.29 [a]	42.66±3.91 [a]	4.12±0.50 [a]
Glibenclamide 5mg/kg bw	10.46±0.91 [b]	85.12±6.91 [b]	11.21±0.81[b]
200 mg/kg bw EELCO&EELPD (1:1)	9.51±0.68 [b]	79.44±5.12 [b]	9.17±0.73 [b]
400 mg/kg bw EELCO&EELPD(1:1)	9.76±0.71 [b]	78.58±5.45 [b]	9.24±0.76 [b]

Each value is mean ± SD for 6 rats in each group. a: $p<0.05$ by comparison with normal rats.
b: $p<0.05$ by comparison with STZ diabetic rats.

In comparison to the normal control group, histopathological examinations of liver sections in control rats revealed that the liver parenchyma was partially deformed structurally and had focal areas of necrosis with crammed blood vessels. Because the EL leaves extract combination of (Dose 200, 400 mg/kg (1:1) produced positive results in the previous study, the investigation was limited to combination treatments. The findings revealed a remarkable appearance of parenchyma with intact architecture and midzonal hepatocytes. **(Fig:3)**. The section of control rat showed normal architecture with islet of Langerhans formed of numerous compactly arranged β - cells, while the section of diabetic rat showed pyknotic nuclei and dark nuclei, with few cells at the periphery having round or ovoid nuclei **(Fig 4)**, while the section of rat treated with EL combination 200 mg/kg b.w showed shrunken nuclei and mild inflammatory cells **(Fig 4c)**. Rats treated with EL combination 400 mg/kg bw had normal architecture with shrunken nuclei **(Fig 4d)** and regeneration of β-cells (70 percent, compared to

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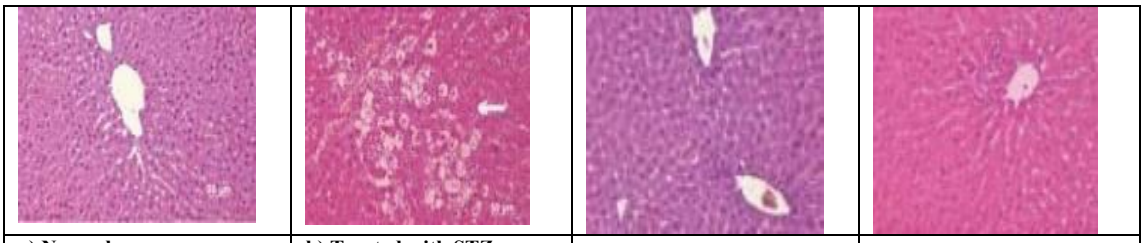
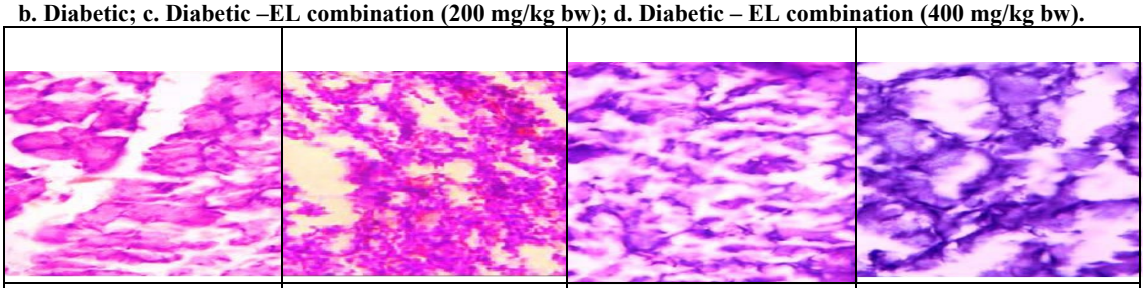
positive control group 30 percent), while the periphery was made up of large -cells (25 percent , compared to positive control group 65 percent). Histopathology studies on the liver revealed that EL has a protective effect. EL treatment preserved the architecture of the liver parenchyma as well as the perivenular, periportal, and midzonal hepatocytes. The central veins and sinusoids appear unremarkably normal in comparison to the positive control group, which had partially distorted liver parenchyma. In a positive control rat, pancreas showed degeneration of islet β- cells and a decrease in islet cell mass. When compared to positive control rats, groups treated with EL showed regeneration of β-cells and an increase in islet mass. Glibenclamide (5 mg/kg) treatment of diabetic rats resulted in β-cell regeneration as well.



a) Normal	b) Treated with STZ	c) EL treated (200mg) (1:1)	d) EL treated (400mg) (1:1)

Fig 3: Histopathological panels of rat Liver prepared from 4 different groups of rats a. Control; b. Diabetic; c. Diabetic –EL combination (200 mg/kg bw); d. Diabetic – EL combination (400 mg/kg bw).



a) Normal	b) Treated with STZ	c) EL treated (200mg)	d) EL treated (400mg)

Fig 4: Histopathological panels of rat pancreas prepared from 4 different groups of rats a. Control; b. Diabetic; c. Diabetic – EL combination (200 mg/kg bw); d. Diabetic –EL combination (400 mg/kg bw).

4] Conclusion:

In conclusion, the phytoconstituents in *C. occidentalis* and *P. dulce* exert promising anti-hyperglycemic effects in STZ-induced β–cell damaged diabetic rats. According to the discussion above, combined plant ethanolic extracts of *C. occidentalis* and *P. dulce* at dose (400 mg/kg) exhibited significant anti-hyperglycemic activity in STZ-induced diabetic rats. These extracts also improved parameters such as body weight and decreased serum liver parameters, suggesting that they could be useful in the treatment of diabetes. Thus, these anti-hyperglycemic agents can be investigated for the development of anti-diabetic lead molecules, and further research into mechanisms of action may result in effective diabetes treatment and control. The study suggests that the combined extract had a synergistic hypoglycemic effect, as evidenced by increased serum insulin levels and decreased serum lipid levels, and thus attribute to the combined plant extracts' therapeutic value in combating the diabetic condition in rats. However, more pharmacological and biochemical research is needed to determine the precise mechanism of hypoglycemic effects of ethanolic leaf extracts of *C. occidentalis* and *P. dulce.*

Conflict of Interest: The authors have no conflict of Interest.

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