RESEARCH ARTICLE

Evaluation of Hypo-glycemic and Anti-oxidant Potential of *Caesalpinia sappan* Leave in STZ Induced Diabetic Rats

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ABSTRACT

In southern Asia, including India, Sri Lanka, and other countries, the stem bark, root, and leaves of *Caesalpinia Sappan* (Fabaceae) have been utilized in traditional medical systems to cure diabetes and numerous other illnesses. The hypoglycemic and hypolipidemic potential of this valued plant's leaves and bark, however, has not yet been proven by science. The current study's objective is to assess the hypoglycemic and hypolipidemic effects of *Caesalpinia Sappan* leaf ethanol extracts. Hydro-alcoholic extracts in dose 200mg/kg of *Caesalpinia Sappan* leaves (CSL) was evaluated for hypo-glycemic effect by *In vitro* α-amylase & *α-glucosidase* anti-diabetic potential similarly *in vivo* by using animals for 21 days in addition to the hypolipidemic potential in rats with diabetes induced by streptozotocin.The α-amylase & *α-glucosidase* inhibition activity was enhanced with enhanced in the concentration of extract. On administration of CSL it restored the body and organs weight. Similarly When CSL extracts were given to diabetic animal as supplements; the levels of GlyHb and plasma glucose were recovered, while insulin, C-peptide, and Hb parameters increased. In the current investigation, MDA levels were shown to be elevated; however, these levels were considerably decreased on the addition of glibenclamide and the hydro-ethanolic extract of CSL.The presence of active principles in the extract and fractions could be the cause of the observed outcome. In comparison to glibenclemide, the usual medication, the results demonstrated the potential effects of *Caesalpinia Sappan* leaves extract as hypoglycemic and anti-hyperlipidemic in a dose-dependent manner. Scientific evidence supports the traditional usage of this substance as an anti-diabetic.

Keywords: *Caesalpinia Sappan*, glibenclemide, leaves, STZ, hypoglycemic.

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INTRODUCTION

Diabetes is a long-term metabolic disorder of the lipid, protein, & carbohydrate metabolism that is characterized by elevated postprandial blood sugar levels and increased very fast. By 2025, the percentage of people worldwide expected to have diabetes would enhance from 4% to5.4%. As per WHO predictions, most of the load will fall on the poorer nation.[1,2] It is estimated that 33 million adults in India suffer from diabetes. This number will most likely increase to 57.2 million by 2025.[2,3] Insulin that is either insufficient or not working properly causes diabetes mellitus, a complex metabolic disease. Damaged pancreatic β cells, oxidative stress, and cardiovascular issues are the hallmarks of chronic hyperglycemia.[4-6] Type II diabetes (T2DM) is a prevalent form of the disease that is thought to be one of the most frequent lifestyle diseases worldwide. There is also a higher prevalence of Type II. Due to its association with chronic complications such retinopathy, nephropathy, neuropathy, skin problems, etc., type 2 diabetes is a non-communicable illness that ranks among the world's top causes of death.[7] Even though synthetic pharmacological therapy of Type II diabetes has made significant progress, research and development of natural anti-diabetic plant products is still ongoing. Numerous hypoglycemic plants are well-known from folklore, but their incorporation into contemporary treatment plans must wait for the creation of an animal testing method that closely mimics the pathogenic progression of diabetes in humans.[8-11] Utilization of plants to improve health & healing or to prevent & manage illnesses and afflictions is known as the manufacture of herbal medications. These are medications derived from herb that are utilized for any of these uses. [12-13] Differentiated herbal medications are given by the WHO as whole, labeled medical goods with active substances, or other plant material or mixtures. The analysis of quality, safety, & effectiveness of natural drugs must adhere to strict rules established by the WHO. According to an estimate, 80% of the world's population today receives substantial medical treatment from herbal medicines. Interestingly, traditional uses of herbal medicines in many nations may also include naturally occurring inorganic or organic active ingredients that do not originate from plants.[14-15] *Caesalpinia sappan* is valued for its medicinal value and is well-known for its anti-inflammatory, antioxidant, and anticancer effects. Numerous bioactive substances,

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Table 1: Drug dosage and grouping for anti-diabetic effects

Group	Drug and Doses
	STZ -Induced diabetes
Group I	Non-Diabetic Control (Saline)
Group II	STZ-only diabetic control group
Group III	*Caesalpinia Sappan* 200 mg/kg b.w. (p.o.)
Group IV	Glibenclamide 0.5 mg/kg

including brazilin, brazilein, sappan chalcone, and protosappanin A, have been found to be present in sappan wood. It has also been found that these compounds offer a number of health benefits; they are anti-oxidants, improve blood circulation, and reduce inflammation. A variety of ailments, including skin disorders, respiratory infections, and gastrointestinal issues, have been treated with sappan wood as a medication.[16-17]

Table 2: Extractive value (in % w/w) in different solvents

S. No	Extracts	CSL % Yield (W/W)
1.	Aqueous extract	16.23
2.	Hydro-Alcoholic extract	25.63
3.	Hexane extract	4.06
4.	Ethyl acetate extract	15.25
5.	Chloroform extract	18.01

MATERIALS AND METHODS

Reagent and Chemicals

STZ and standard drug were purchased from Aventis Pharma ltd. Chemical kits for estimation of various parameters were taken from local market. All other reagents of analytical grade were obtained from Merck Chemicals.

Plant Material

Table 3: Examining the solvent fractions and crude extract phytochemically

S. No.	Test	Observation	Inference
1	Alkaloids		
	Mayer's test	White PPT	---
	Dragendorff's test	Orange PPT	---
	Hager's test	Yellow PPT	---
	Wagner's test	Reddish brown PPT	---
2	Glycosides		
	(i)Cardiac Glycosides		
	Keller Kiliani test	The upper surface is bluish-green, while the confluence of the two liquid layers is reddish brown in color.	+++
	Legal's test	Pink	+++
	Baljet's test	Yellow	+++
	(ii)Saponin Glycosides		
	Foam test	Observation of persistant foam	---
3	Carbohydrates		
	Molish's test	Violet ring formation at the junction	+++
	Fehling's solution A & B	Yellow then red precipitate formation	+++
4	Steroids		
	Leibermann-Burchard reaction	The colors red, blue, and green come first.	+++
	Salkowski reaction	Greenish fluorescence in the acid layer and red coloration in the chloroform layer	+++
6	Phenolic compounds & tannins		
	$FeCl_3$ solution test	Dark blue color	+++
	Gelatin solution test	White PPT	+++
	Lead acetate solution test	White PPT	+++
7	Proteins & Amino acids		
	Millon's test	Red precipitate is created when white precipitate dissolves or turns brick red.	+++
	Biuret test	Violet or pink colour	+++
	Xanthoprotein test	After boiling, the white precipitate creation turns yellow, and when ammonium hydroxide is added, it finally turns orange.	+++
	Ninhydrin test	Purple or bluish colour	+++
8	Flavonoids		
	Sulphuric acid test	Orange, Yellow, Red colour	+++
	Lead acetate solution	Yellow precipitate	+++

Table 4: in vitro alpha amylase potential of the ethanol extract of CSL

% of Inhibition	Conc (µg/ml)					
	100	200	300	400	500	IC_{50} (µg/ml)
CSL	10.77±0.75	19.42±1.35	45.30±3.17	57.55±4.02	76.34±5.34	347.93
Std. (Acarbose)	23.86±1.67	39.28±2.74	57.55±4.02	72.65±5.08	91.44±6.40	258.77

Values expressed as Mean ± SD for triplicates

Table 5: in vitro alpha glucosidase potential of the ethanol extract of CSL

% of Inhibition	Conc (µg/ml)					
	100	200	300	400	500	IC_{50} (µg/ml)
CSL	13.09±0.91	24.90±1.74	36.34±2.54	60.70±4.24	72.34±5.04	354.55
Std. (Acarbose)	24.90±1.74	44.83±3.13	62.36±4.36	76.56±5.35	92.44±6.40	238.85

Values expressed as Mean ± SD for triplicates

Gathering and Verifying the Species

Leaves fully grown plants of *Caesalpinia Sappan L*. (CS) are collected from south region, Tirunelveli, TN in September. The specimen identified & verified by Dr. S. Muthesswaran, scientist of "Xavier Research Foundation", Palayamkottai, Tamil Nadu, India (XCH40426) and also authenticated from Dr. K.N. Sunil Research Officer, SCRI, Arumbakkam, Tamil Nadu, with voucher specimen (PCOGOO2-ACF) Certificate no. 331.18072201.

Extraction of Drug

For the extraction, standard protocol and analytical grade solvents were employed. 40 g of powdered plant part (leaves) were first extracted using a Soxhlet apparatus with chloroform and n-hexane, and then using the same process, the plant parts were extracted with a hydroalcoholic solvent at a temperature of 50–65ºC

Phytochemical screening of extract

Using established techniques, the presence or lack of secondary metabolites in the crude extract was determined by screening such as flavonoids, saponins, anthraquinones, phenolic compounds, alkaloids, reducing sugars, and cardiac glycosides. The capacity of phenolic compounds to scavenge free radicals, or bind metal cations is what gives them their antioxidant action.[18-20]

***In vitro* α-amylase anti-diabetic potential**

α-amylase suppression investigation conducted *in vitro* as per standard procedure Using phosphate buffer (pH 6.8) (PBS), extracts of leaves of *Caesalpinia sappan* were produced in various concentrations from 100µg/ml to 500µg/ml. For 10 minutes, 500µl of 20% (v/v) plant extract and 500µl of 20mM PBS with 0.5mg/ml of α-amylase were incubated at 25°C. 1000µl of 0.5% starch in 20 mM PBS, was added following the pre-incubation period. After that, the reaction solution was incubated for 10 min at 25°C. 500µl of 96mM 3, 5-dinitrosalicylic acid color chemical was used to stop the reaction. The test tubes were incubated in a bath of boiling water for five minutes, and then they were allowed to cool. At 540 nm, absorbance (A) was measured. Using acarbose as a positive control, the percentage of inhibition and α-amylase's inhibitory activity was computed as follows: (Control O.D. - Test O.D.) / Control O.D. × 100 equals the percentage of inhibition.[21]

***In vitro* α-glucosidase inhibition study**

5-millimolar para-nitrophenyl-α-D-glucopyranoside After 10 min of incubation at 25°C, 50µl of the various concentrations (10µg/ml to 100µg/ml) of *Caesalpinia sappan* leaves extract and 100µl of yeast α-glucosidase solution were added. The mixture was then diluted with 0.1mol phosphate buffer (pH 6.9). After five minutes of incubation at 25°C, the reacting liquid was measured for absorbance at 405 nm. Using acarbose as a positive control, calculation done as above.[22]

Animals

Table 6: Effect of CSL on animal body weight

S. No	I	II	III	IV
Initial day (gm)	186.66±2.88	185.00±5.00	183.33±5.77	188.33±5.77
Final day (gm)	221.66±2.88	148.33±7.63	185.00±5.00	215.00±5.00

Table 7: Effect of CSL on animal organ wt. in control & experimental rats

Parameter	I	II	III	IV
Liver (gm)	6.50±0.06	8.51±0.51	7.86±0.12	6.51±0.09
Kidney (gm)	1.30±0.05	1.75±0.01	1.65±0.01	1.31±0.01
Pancreas (gm)	0.84±0.01	1.12±0.06	1.04±0.02	0.85±0.01

The values for six rats are given as Mean ± SD

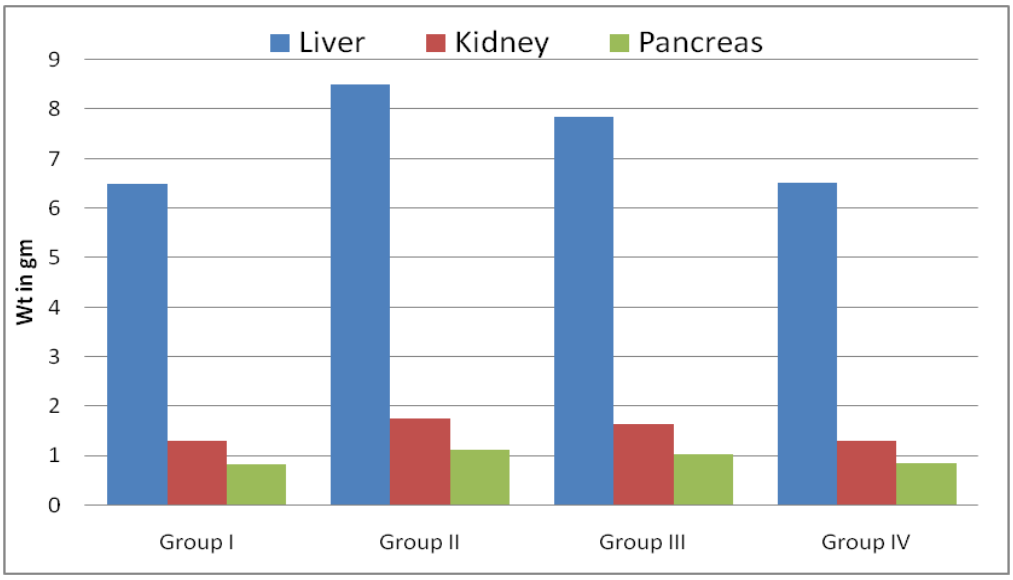

Fig.1 Effect of CSL on animal body organ

Prior to the experiment, the institutional Animal Ethics Committee provided ethical clearance (CPCSEA registration number: 1659/PO/a/ CPCSEA).

Hypoglycemic potential of leaves extract on STZ - induced diabetes model

Preparation of diabetic rats

A single IP injection of 60 mg/kg b.w. STZ was used to produce DM in adult male Wistar albino rats that had been fasted for the previous night. According to Murali et al. (2002), the injection volume was produced to contain 65 mg/mL and administered as 0.1 mL/100 g of b.w.. After five days, hyperglycemic (>210 mg/dL blood glucose level BGL) mice were taken for the study, and BGL were assessed using a glucometer.[23]

Animal groups & doses

Samples Collection

After work, the experimental animals were given a 12-hour fast with no water restrictions, and their orbital vein plexus was used to take blood samples from rats that had been anesthetized with diethyl ether.

Histopathological estimation

Pancreas, kidney, and liver tissues were promptly removed from each experimental group for histological analysis. Liver, kidney, and pancreatic tissues were kept in formaldehyde 10% buffered. Following standard procedures, the paraffin-embedded preserved tissues were cut into 4 µm thick sections, & placed on slides for staining with hematoxylin and eosin. Light microscopes were used to examine every segment.[24]

Statistics

Every value is given as Mean ±SEM. Tukey's multiple comparison tests were conducted after one way analysis of variance (ANOVA) was used to compare the differences. P-values less than 0.05 were regarded as noteworthy.

RESULTS

Extraction of Drug

After extraction the *Caesalpinia sappan* leaves (CSL) crude extract shows highest extract in Hydro-Alcoholic solvent and it was 25.63% and yield in different solvent was 16.23 %, 15.25 and 18.01 for Aqueous, Ethyl acetate and chloroform respectively (Table 2).

Phytochemical screening of extract

For the Hydro-Alcoholic extract of leaves, a preliminary phytochemical study was performed and the resulted indicates that the major phytochemicals present in the extracts were, glycoside, flavonoids, phenolic compounds, protein, amino acid and tannins (Table 3).

Inhibition of alpha amylase activity

The α-amylase inhibition potential enhanced with enhanced in the amount. The half inhibition concentration (IC_{50}) of *Caesalpinia Sappan* (powder of leave-CSL) and acarbose were 347.93, and 258.77 µg/ml respectively (Table 4). The lowest IC_{50} value has greatest inhibition activity.

Alpha glucosidase activity inhibition

The α-glucosidase inhibition potential is directly proportional to the concentration. The half inhibition concentration (IC_{50}) of *Caesalpinia Sappan* (CSL) and

Table 8: Effect of CSL on glucose homeostasis

Parameter	I	II	III	IV
Glucose (mg/dl)	83.53±3.50	253.88±8.77	158.59±5.89	87.83±3.94
Hb (gm/dl)	14.41±0.14	8.65±0.27	10.82±0.24	14.17±0.15
GlyHb (% Hb)	6.51±0.13	13.70±0.14	9.33±0.12	6.55±0.14
Insulin (µU/ml)	15.44±1.10	5.27±0.14	9.81±0.14	15.31±0.28
C peptide (µU/ml)	256.51±12.11	129.95±11.50	185.87±8.72	248.07±17.09

The values for six rats are given as Mean ± SD. using SPSS version 24 for statistical analysis of one-way ANOVA, and DMRT test. Gp I: NC; Gp II: DC (STZ only);
Gp III: CSL extract, Gp IV: STZ + Glibenclamide (Std. Drug)

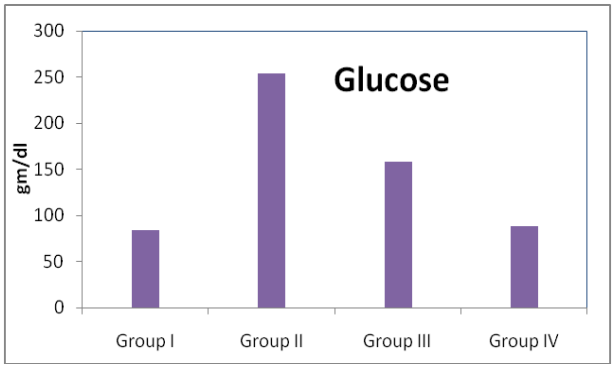

Fig.2 Effect of CSL on Glucose

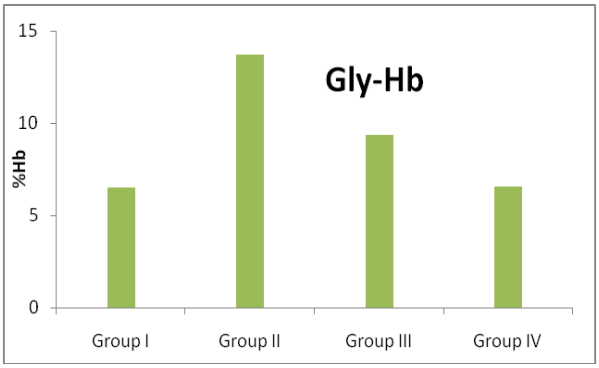

Fig. 3 Effect of CSL on GlyHb

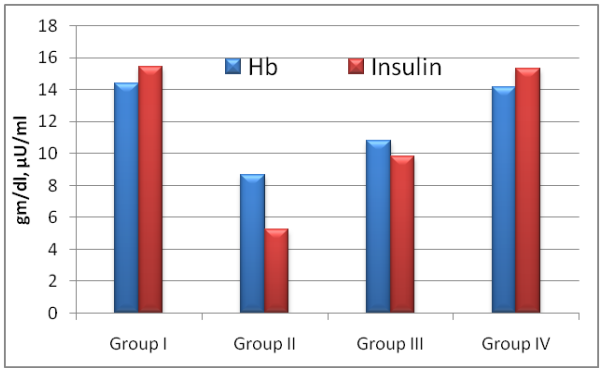

Fig. 4 Effect of CSL on Hb and Insulin

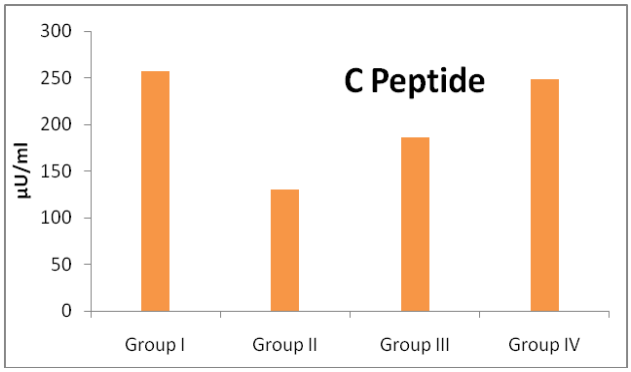

Fig.5 Effect of CSL on C – peptide

acarbose were 354.55, and 238.85µg/ml respectively (Table 5). The lowest IC_{50} value has highest inhibition activity.

Effect of *Caesalpinia Sappan* Leaves on body & weight of organ in control & experimental animal

The Streptozotocin treated diabetic rats shows significant decreased in body weight while increased organs weight was observed in diabetic rats. On supplementation of *Caesalpinia Sappan* leaves restored the body and organs weight (Table 6, 7, Fig. 1). Significant activity was observed and nearest to the standard Glibenclamide. In people with diabetes mellitus, proteins are not able to be used by body cells as a source of energy and proteins are instead used as energy sources. As a result, body weight decreases and there is less protein stored. It is commonly known that DM results in an inability to use glucose for energy, which increases the use of protein and decreases its storage. This changes the weight of the body and its organs, including the liver, kidney, & pancreas, primarily through the depletion of body proteins.[25]

Effect of CSL on glucose homeostasis in control and experimental rats

In the current work, experimental rats indicates higher levels of plasma glucose, GlyHb, & Hb & lower levels of insulin, C-peptide, and Hb in comparison to normal control rats. When CSL extracts were given to diabetic animal as supplements, the levels of GlyHb and plasma glucose were recovered, while insulin, C-peptide, and Hb parameters increased (Table 8, Fig. 2-5).

Restorative effect of CSL extract on plasma lipid profile

LDL-C, VLDL-C, plasma cholesterol, phospholipids, & modest amounts of HDL-C were all greater in diabetic rats. Following the injection of CSL extract, there was a significant restoration of all above factors (Table 9).

The current investigation found that supplementing with extracts raised HDL content and lowered LDL, TC, VLDL, and TC content in a dose-dependent way. While increasing HDL content, the standard treated group also saw decreases in LDL, VLDL, FFA, and TC. As previously noted, elevated blood cholesterol levels are linked to elevated amounts of free fatty acids in diabetes mellitus.[26] The fractions of cholesterol, such as VLDL, LDL, and HDL, represent the total cholesterol.

Effect of CSL on glucose metabolism in control and experimental rats

The current work investigate the effects of CSL extracts on the activity of the main enzymes involved in the metabolism of glucose in diabetic rats, including glucose kinase, G 6-P dehydrogenase, G 6-P, & F-1,6-bis phosphatase. Compared to normal group, diabetic rats showed higher activities of glucose kinase, G-6-P, and F-1,6-bisphosphatase & lower activities of G-6-P dehydrogenase & glycogen content (Table 10). On supplementation of extracts restored the glucose metabolizing enzymes. Our findings are in line with previous research by Rajiv Gandhi and Sasikumar (2012), which found that administering *M. emarginata* remarkably decreased the effect of G-6-P, F-1,6-bisphosphatase while significantly increasing those of enzymes that metabolize carbohydrates, such as hexokinase. NADPH is produced by G-6-P dehydrogenase when it uses glucose and the pentose phosphate pathway. For GSH synthesis & free radical scavenging, NADPH is required. The active ingredients in the CSL extract may have improved glucose consumption

Table 9 Effect of CSL on plasma Lipid profile

Parameter	I	II	III	IV
Phospholipids (mg/dl)	160.10±8.86	241.42±9.07	212.43±9.77	164.51±7.16
Triglycerides (mg/dl)	76.05±3.18	188.55±4.85	123.83±5.60	78.96±3.94
Cholesterol (mg/dl)	128.85±7.57	252.16±10.29	199.82±9.94	137.28±7.36
HDL (mg/dl)	45.13±2.86	27.45±2.09	34.44±1.65	44.71±1.60
LDL (mg/dl)	68.51±10.69	187.00±9.53	140.61±8.89	76.77±7.21
VLDL (mg/dl)	15.21±0.63	37.71±0.97	24.76±1.12	15.79±0.78

The values for six rats are given as Mean ± SD. using SPSS version 24 for statistical analysis of one-way ANOVA, and DMRT test.

Table 10 Effect of CSL on liver glucose metabolism activity in rats

Parameter	I	II	III	IV
Glucokinase (µmol of glucose phosphorylated /h/mg protein)	0.35±0.03	0.74±0.03	0.58±0.02	0.36±0.04
G-6–phosphate dehydrogenase (1×10^{10}mlU/mg protein)	5.69±0.19	2.23±0.19	3.71±0.16	5.60±0.18
G-6-P (µmoles of Pi liberated/h/mg protein)	1124.66 ±48.42	1737.33 ±45.76	1529.33 ±51.69	1153.33 ±55.07
Glycogen (mg/100g tissue)	54.44±2.83	30.61±2.87	39.34±1.46	52.26±3.04
F-1,6-bisphosphatase (µmoles of Pi liberated/h/mg protein)	523.23±18.27	868.42±28.34	662.42±12.34	530.80±11.53

Table 11 Effect of CSL on serum anti-oxidants

Parameter	I	II	III	IV
MDA (nmol of MDA formed/L)	7.35±0.08	16.76±0.14	11.47±0.09	7.44±0.09
SOD (U/ml)	4.58±0.09	3.02±0.03	3.27±0.10	4.56±0.12
Cat. (U/ml)	10.44±0.08	7.21±0.13	8.68±0.07	10.42±0.13
GPx (U/ml)	8.75±0.12	5.83±0.10	6.66±0.12	8.73±0.07
GSH (mg/dl)	8.18±0.17	5.12±0.14	6.83±0.06	8.10±0.10
Vit-C (mg/dl)	4.73±0.11	2.77±0.13	3.77±0.10	4.66±0.12
Vit - E (mg/dl)	3.82±0.07	2.17±0.08	2.71±0.11	3.81±0.07

Table 12 Effect of CSL on Liver anti-oxidants

Parameter	I	II	III	IV
MDA (nmoles/mg Tissue)	10.59±0.13	17.80±0.13	14.26±0.07	10.62±0.04
SOD (U/ mg Tissue)	3.64±0.11	2.43±0.06	2.19±0.03	3.63±0.06
Cat. (U/ mg Tissue)	4.33±0.06	2.15±0.08	3.17±0.05	4.29±0.11
GPx (U/ mg Tissue)	8.72±0.08	5.21±0.09	6.62±0.09	8.70±0.08
GSH (µg/ mg Tissue)	6.61±0.09	4.15±0.10	5.64±0.12	6.59±0.10
Vit-C (µg/ mg Tissue)	5.46±0.16	2.87±0.08	3.91±0.05	5.41±0.08
Vit - E (µg/ mg Tissue)	4.06±0.07	2.23±0.10	3.18±0.03	4.02±0.04

by raising the activity of G-6-P dehydrogenase through an activated PPP pathway. They do this by enhancing the peripheral consumption of glucose, reversing the impairment in liver glycolysis, and restricting the generation of gluconeogenic glucose, which is similar to insulin.[27]

Effect of CSL on serum antioxidant status

Malondialdehyde (MDA), antioxidant enzymes like SOD, CAT, and GPx, & non-enzymatic antioxidants like glutathione (GSH), vit C, & vit E in Streptozotocin in animals were all examined in the current study. Compared to normal rats, diabetic rats had higher levels of malondialdehyde (MDA), lower levels of antioxidant enzymes like SOD, CAT, & GPx, as well as lower levels of nonenzymatic antioxidants such glutathione (GSH), Vit C, and Vit E. Based on these findings, it was hypothesized that the CSL extracts had neutralized oxidative stress. In the current investigation, MDA levels were shown to be elevated; however, these levels were considerably decreased upon the addition of glibenclamide and the hydroethanolic extract of CSL (Table 11). These show that the anti-peroxidative properties of the components in the ethanol extract of CSL prevent oxidative damage. According to this work, SOD is crucial for removing ROS produced during

Table 13 Effect of CSL on Kidney anti-oxidants

Parameter	I	II	III	IV
MDA(nmoles/mg Tissue)	5.25±0.10	8.57±0.35	7.17±0.12	5.30±0.04
SOD (U/ mg Tissue)	1.76±0.12	0.94±0.04	1.15±0.03	1.75±0.07
Cat. (U/ mg Tissue)	2.22±0.06	1.12±0.05	1.35±0.09	2.20±0.04
GPx (U/ mg Tissue)	4.36±0.05	2.12±0.06	3.14±0.08	4.33±0.06
GSH (µg/ mg Tissue)	3.30±0.06	2.24±0.13	2.57±0.11	3.28±0.07
Vit-C (µg/ mg Tissue)	2.70±0.19	1.45±0.08	1.83±0.10	2.68±0.11
Vit - E (µg/ mg Tissue)	2.24±0.09	1.08±0.02	1.38±0.12	2.19±0.19

Table 14: Effects of CSL on DPPH and H_2O_2 Scavanging Potential

Concentration (µg/ml)	Percentage inhibition (I)			
	DPPH assay		H_2O_2 assay	
	CSL	Ascorbic acid	CSL	Ascorbic acid
20	24.87	52.86	27.52	49.35
40	41.18	55.51	33.93	57.00
60	47.33	58.30	39.98	60.63
80	52.67	61.67	44.88	64.46
100	60.43	68.58	48.71	69.40
IC_{50} (µg/ml)	71.42	10.19	101.50	17.34

the peroxidative breakdown of STZ xenobiotics. An essential part of the antioxidant defense system is CAT.

Effect of CSL on liver & kidney antioxidant status in control & experimental rats

The increased content of malondialdehyde (MDA), decreased activities of antioxidant enzymes, like SOD, CAT, GPx & non-enzymatic like GSH, Vit C & Vit E in liver & kidney of diabetic rats comparison with normal rats (Table 12, 13). On supplementation of CSL extracts restored the antioxidants potential.

Antioxidant activity: DPPH Assay

All samples indicate dose dependent effect on the DPPH scavenging potential. As the concentration increased the absorbance decreased which was compared to the absorbance of control that was ascorbic acid. Percentage inhibition was calculated of each concentration and a graph plotted between concentration and percentage inhibition (Figure 14 and Fig. 6). At highest concentration of CSL the percentage inhibition was found to be 60.43%. Ascorbic acid indicates % inhibition of 68.58% at the highest conc which is lower than the sligh more than CSL. The concentration of the extract required for 50% inhibition is called IC_{50} and was found to be 71.42 and 10.19 µg/ml for CSL and ascorbic acid, respectively.

Hydrogen peroxide scavenging assay

Similar to the DPPH assay, extracts produced dose dependent effect on the hydrogen peroxide scavenging activity. As the concentration increased, the absorbance decreased which was compared to the absorbance of control that was ascorbic acid. Percentage inhibition was calculated of each concentration and a graph plotted between concentration and percentage inhibition. Standard produced % inhibition of 69.40% at the highest concentration. At highest concentration of CSL the percentage inhibition was found to be 48.71%. IC50 was found to be 101.50 and 17.34 µg/ml for CSL and ascorbic acid, respectively.

DISCUSSION

The results obtained from the extractive value in the pharmacognostical evaluation indicates the presence of more polar compounds as the higher extractive value was found in water & alcohol as solvents hence the aqueous-alcoholic (hydroalcoholic) solvent was selected for extraction. Alkaloids, flavonoids, saponins, phenols,

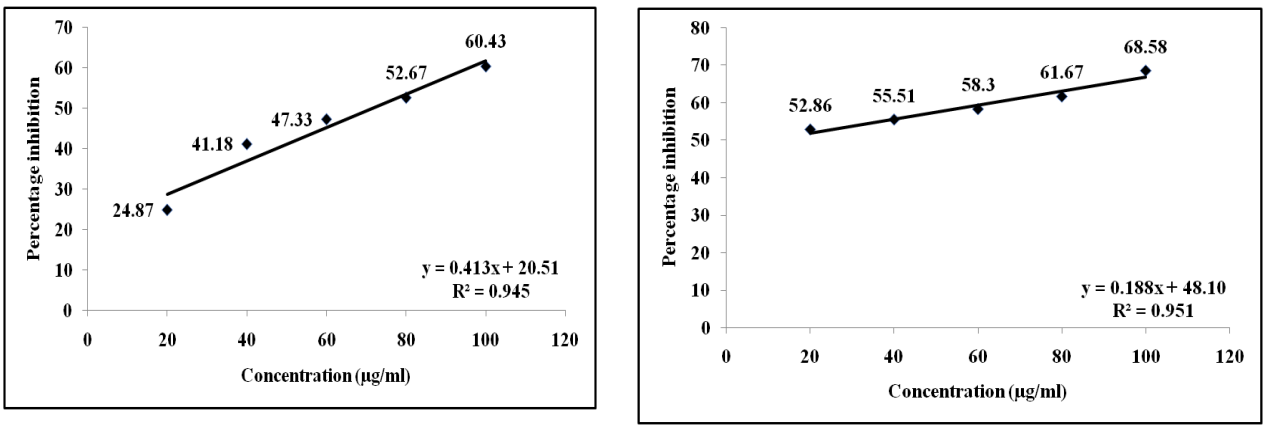

Figure 6: Percentage inhibition of DPPH by CSL and by ascorbic acid

tannins, and carbohydrates are found in the ethanol crude extract of *Caesalpinia Sappan*, according to a preliminary phytochemical investigation. These compounds are known to have anti-oxidant, hypoglycemic, and hypolipidemic properties. [28] So the present work analyzed the anti-diabetic potential of the ethanol crude extract and solvent fractions of *Caesalpinia Sappan* leaves due to presence of the phenolic and flavonoids compounds. Excessive oxidative stress causes lipids and proteins to oxidize, changing their structure and function in the process. Consequently, antioxidant principles or agents that scavenge free radicals may be included in prospective antioxidant therapy. Finding natural compounds with antioxidant qualities is now the main emphasis, and this needs more research. Diabetes mellitus has been linked to dehydration and weight loss.[29] There was a decrease in body weight & an increase in food consumption in experimental animal. This suggests that the patient is polyphagic and has lost weight as a result of increased tissue protein breakdown.[30] When comparing the body & organ weights of the animals treated with CSL hydroethanolic extract at different doses to those of animals with diabetes, a substantial rise was observed. When extracts were added to diabetic rats, the weight of their bodies and organs was recovered. The extracts showed signs of anti-diabetic action. This might be because the diabetic rats' hypoglycemia state was better managed.[31] An essential part of the metabolism of fats is played by insulin. Insulin is a strong lipolysis inhibitor. Due to the fact that it prevents the production of free fatty acids & prevent the activity of hormone-sensitive lipases in adipose tissue. When fatty acid concentrations are raised, fatty acids are also more readily beta-oxidized, which results in higher levels of cholesterol and acetyl CoA in diabetics. Major reason of CHD is accumulation of triglycerides. One well-known effect of proven diabetes mellitus is hyperlipidemia. It has been documented that diabetic rat experience hypertriglyceridemia and hypercholesterolemia.[32]

CONCLUSION

The study's findings indicate that the polyphenols found in *Caesalpinia sappan's* leaves, including flavonoids and tannins, may be the cause of the plant's hypoglycemic and hypolipidemic effects. The study's findings also point to a considerable reduction in blood glucose levels in both normoglycemic and diabetic rats. Overall, the experimental studies concluded that *Caesalpinia sappan*, Linn. leaves extracts have potential antidiabetic & antioxidant activity.

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