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Phytochemical Screening, *Invitro* Antioxidant and *In-vivo* Antidiabetic Activity of Methanolic Extract of Leaves of *Celastrus paniculatus*

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Abstract

Celastrus paniculatus Willd, commonly known as Staff tree, is a plant species from the Celastraceae family. It contains various active constituents, including celapanigin, malkangunin, celapanin, and zeylasteral, which contribute to its medicinal properties. The objective of this study was to evaluate the qualitative and quantitative phytochemical analysis, in vitro antioxidant activities, and in vivo anti-diabetic potentials of the methanolic extract of C. paniculatus leaves using a streptozotocin-induced rat model. The in vitro antioxidant activity of the methanolic extract was assessed using DPPH and ABTS radical cation decolorization assay methods. Rats were induced with diabetes using streptozotocin and treated with glibenclamide as the standard medication. Body weight and blood sugar levels were measured throughout the study. Phytochemical analysis revealed the presence of alkaloids, glycosides, proteins and amino acids, flavonoids, tannins and phenolic compounds, saponins, triterpenoids, and steroids in the leaves. The total phenolic content of the methanolic extract was 32.33 mg/g, followed by flavonoids at 11.66 mg/g. Oral treatment with the methanolic extract of C. paniculatus at doses of 200 and 400 mg/kg significantly reduced blood glucose levels in diabetic rats compared to control rats ($p < 0.001$) and increased body weight. The chemical constituents of the plant extract may have potential in preventing diabetic complications and could serve as an alternative in the current range of antidiabetic drugs. Further research is recommended to validate the use of this plant as an antidiabetic agent.

Keywords: Celastrus paniculatus, Celastraceae, Diabetes mellitus, phytochemical analysis, Streptozotocin, Glibenclamide.

Introduction

Natural products derived from plants have been widely used as supplements to combat various serious diseases in developing countries [1]. Reactive oxygen species and free radicals are well-known contributors to molecular, cellular, and tissue pathogenesis, leading to several health risks such as atherosclerosis,

arthritis, cardiovascular diseases, central nervous system injuries, gastritis, cancer, aging, and Acquired Immune Deficiency Syndrome (AIDS) [2-5]. Considering the lack of effective therapies and the role of oxidative damage in chronic conditions, the use of antioxidants to protect against these diseases is justifiable [6,7]. Antioxidants have the ability to delay or inhibit oxidation processes caused by atmospheric oxygen or reactive oxygen species. Both enzymatic antioxidants like superoxide dismutase, catalase, glutathione peroxidase, and non-enzymatic compounds such as uric acid, bilirubin, and albumin possess endogenous antioxidative potential [8,9]. Natural antioxidants often exhibit stronger biological activity compared to many synthetic antioxidants, which may have potential carcinogenic effects [10,11].

Diabetes mellitus is the leading cause of blindness, renal failure, strokes, lower limb amputations, and heart attacks among adults worldwide. According to the International Diabetes Federation, the number of people with diabetes is projected to reach 330 million by 2025, with Africa and Asia experiencing the largest increases. Developing countries are expected to account for over 75% of diabetes cases by 2025, compared to 62% in 1995 [12]. Current treatments for diabetes include insulin and oral antidiabetic drugs such as glinides, biguanides, and sulfonylureas [13]. It has been reported that certain orally consumed herbs and plants possess hypoglycemic properties [14]. The World Health Organization (WHO) recognizes over 1200 plant species worldwide that are used in the treatment of diabetes mellitus [15]. Many plant species contain alkaloids, terpenoids, glycosides, carotenoids, flavonoids, and other compounds known for their antidiabetic properties [16]. Exploring the hypoglycemic, antioxidant, and hypolipidemic qualities of plants may lead to the development of new pharmaceutical treatments for diabetes mellitus [17].

Streptozotocin (STZ) is a commonly used diabetogenic drug. It is an antibiotic derived from Streptomycetes achromogenes and is inexpensive with minimal side effects [19]. STZ, in combination with nicotinamide (NA), is used to induce both type 1 and type 2 diabetes [20]. STZ exerts its damaging effects through various mechanisms, including the generation of reactive oxygen species (ROS), nitric oxide, NF-B, reduction of mitochondrial membrane potential, and activation of c-Jun N-terminal kinase [21].
C. paniculatus, also known as Malkangani, Jyotishmati, or the memory-enhancing plant, has been traditionally used to treat various human ailments such as dysentery, diarrhea, fever, and digestive disorders like loss of appetite, constipation, and abdominal distension [22]. The dried powdered form of its leaves, flowers, fruits, and seeds is recommended for regular consumption to improve mental disorders and enhance mental power. Additionally, boiled leaves are externally used to treat swellings and fractures [23]. The leaves of C. paniculatus are rich in secondary metabolites such as proteins, polyphenols, carbohydrates, flavonoids, and terpenoids [24]. This study aims to evaluate the qualitative and quantitative phytochemical analysis, in vitro antioxidant activities, and antidiabetic potential of the methanolic extract of C. paniculatus leaves in diabetic rats.

Materials and methods

Plant material

The leaves of C. paniculatus were obtained from Pinnacle Biomedical Research Institute (PBRI) located at Near, Bharat Scout and Guides Campus, Shanti Marg, Shyamla Hills Road, Bhopal, Madhya Pradesh 462003, India. The plant's identification and authentication were conducted by Dr. Saba Naaz, a botanist from the Department of Botany at Saifia College of Science in Bhopal. To serve as a reference for future use, a voucher specimen numbered 189/Saif./Sci./Clg/Bpl. was stored in the Department of Botany, Saifia College of Science, Bhopal.

Chemical reagents

The chemicals utilized in this study were provided by various suppliers. Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India), Hi Media Laboratories Pvt. Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India) were the sources of the chemicals. Glibenclamide was obtained from Unichem Ltd. (Alkem, Mumbai). Streptozotocin was used as well. Only analytical-grade substances were employed in the research.

Extraction

Plant material fattening

The plant material obtained from C. paniculatus was crushed and dried naturally at room temperature. Soxhlet extraction method was employed to extract the components from the dried plant material using petroleum ether after coarse grinding. The extraction process was repeated several times until sufficient extraction was achieved.

Extraction by soxhlation process

The defatted powder of C. paniculatus was subjected to thorough extraction using methanol through the Soxhlet extraction method. The extract was concentrated by evaporation under controlled conditions, ensuring that the solvent was completely removed. The resulting dried and concentrated extract was weighed to determine the extractive yield. Prior to analysis, the extract was transferred into glass vials measuring 6 x 2 cm and stored at a temperature of 4°C in a refrigerator [25].

Phytochemical screening

Phytochemical screening was conducted following established protocols [26, 27] to identify potential bioactive compounds. The screening involved the observation of color changes or the formation of precipitates upon the addition of specific reagents to the solution, indicating the presence of certain compounds.

Total phenol measurement

The total phenolic content of the extracts was determined using the Folin-Ciocalteu reagent. A standard solution of gallic acid (20-100 µg/ml) was prepared in CH3OH. Plant extract solutions at a concentration of 100 µg/ml were also prepared in CH3OH. To each test tube, 0.5 ml of the sample and 4 ml of 7.5% sodium carbonate were added, followed by 2 ml of a 10-fold diluted Folin-Ciocalteu reagent. The tubes were covered and incubated at room temperature (RT) for 30 minutes with periodic shaking. The absorbance at 765 nm was measured against an empty CH3OH reference. The total phenolic content was determined using the calibration curve of gallic acid, and the results were expressed as milligrams per gram (mg/g) of gallic acid [28].

Total flavonoids measurement

Rutin solutions were prepared at various concentrations (20 to 100 µg/ml) in CH3OH. Test samples with a similar polarity and concentration of 100 µg/ml were prepared. A 0.5 ml aliquot of the sample was diluted and added to 0.15 ml of a 5% NaNO2 solution, followed by 2 ml of distilled H2O. After 6 minutes, a 10% AlCl3 solution was added, and the mixture was allowed to stand for 5 minutes. Then, 2 ml of a 4% NaOH solution was added. The final volume was adjusted to 5 ml with distilled water, and the mixture was left to stand for an additional 15 minutes. The absorbance at 510 nm was measured using distilled water as the reference. The total flavonoid content was determined using the calibration curve of rutin [29].

Antioxidant activity

DPPH radical scavenging activity

The DPPH assay was performed following the method described by Gulçin et al. (2006) [30]. A solution of 0.1 mM DPPH (4 mg/100 ml) in methanol was prepared. Then, 1 ml of this DPPH solution was mixed with 1 ml of different concentrations of the extracts. The reaction mixture was thoroughly vortexed and kept in the dark at room temperature for 30 minutes. Ascorbic acid was used as a reference standard, and methanol was used as a control. The reduction of the stable DPPH radical served as an indicator of the antioxidant capacity of the C. paniculatus extracts. The change in color was measured at a wavelength of 517 nm using a methanolic solution as the reference. The absorbance of the control without the plant extracts was used for comparison. The percentage inhibition of the free radical DPPH was calculated using the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. All the tests were conducted in triplicates. The antioxidant activity was expressed as the 50% inhibitory concentration

(IC50), which was calculated based on the percentage of DPPH radicals scavenged. A lower IC50 value indicates higher antioxidant activity.

ABTS radical cation decolourization assay

A stock solution of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) was prepared by dissolving it in water at a concentration of 7 mM. To induce incomplete oxidation of ABTS, the stock solution was mixed with 2.45 mM potassium persulfate and allowed to stand in the dark at room temperature for 12-16 hours. This resulted in the formation of a stable ABTS radical that remained in this form for more than two days under appropriate storage conditions (in the dark at room temperature).
For the assay, the incubation mixture was prepared with a total volume of 5 ml, which included 0.54 ml of the ABTS solution, 0.5 ml of phosphate buffer, and various concentrations of the C. Paniculatus leaf extracts (20, 40, 60, 80, and 100 µg/ml). The blank sample consisted of water instead of the sample or the standard. The absorbance of the mixture was measured spectrophotometrically at 734 nm and compared with a standard (ascorbic acid) [31].

Animals

Wistar rats weighing approximately 200±50 g were housed in groups of six, with a total of 12 rats (n=12), in a controlled environment with regulated humidity and temperature (25±2 °C, 55-65%). The rats were provided with standard rodent food and had unlimited access to water throughout the study. Prior to the commencement of the experiments, a period of 7 days was allotted for the rats to acclimate to the laboratory conditions. All experiments were conducted between 8:00 and 15:00 hours in a noise-free room. Each set of experiments involved a separate group of rats consisting of six individuals (n=6). The Institutional Animal Ethics Committee (IAEC), authorized by India's Ministry of Environment and Forests in New Delhi to oversee and monitor the use of experimental animals, granted approval for the animal experiments.

Streptozotocin (STZ) induced diabetes

A solution of streptozotocin (STZ) with a concentration of 10 mg/ml was prepared in ice-cold 0.1 M citrate buffer at pH 4.5. The rats were administered STZ intraperitoneally at a dose of 50 mg/kg body weight within a span of 5 minutes. The rats used in the experiment were selected based on the presence of moderate diabetes, glycosuria, and hyperglycemia, indicated by blood glucose levels ranging from 200 to 300 mg/dl, 48 hours after receiving STZ. Blood samples were collected from the retro-orbital vein of the rats during the study. Fasting blood glucose levels were measured on day 0, 3, 7, 14, and 21 of the study using the Accu-Check Active glucometer (Roche Diagnostics, Germany). The blood glucose levels were expressed in mg/dl, and the glucometer provided sufficient sensitivity for accurate analysis with only a small amount of blood (1-2 µL) required. The blood samples were collected from the retro-orbital vein and placed on the glucose test strip of the glucometer [32].
For the current investigation, 5 groups of rats, each containing 6 animals, were used as follows:
Group-I: Normal Control - treated with normal saline.
Group-II: Negative Control - Streptozotocin-induced diabetic rats treated with streptozotocin (50 mg/kg in 0.1 M sodium citrate).
Group-III: Standard - STZ-induced diabetic rats treated with Glibenclamide (5 mg/kg) orally.
Group-IV: Test-1 - STZ-induced diabetic rats treated with 200 mg/kg of C. paniculatus.
Group-V: Test-2 - STZ-induced diabetic rats treated with 400 mg/kg of C. paniculatus.

Diabetes was induced by a single intraperitoneal injection of streptozotocin at a dose of 50 mg/kg body weight. Except for Group I, all the other 4 groups were induced with diabetes. Glibenclamide was suspended in 0.9% NaCl in warm water as a vehicle solution and administered orally for 21 days. The treatment schedule commenced on the 4th day after diabetic induction and was considered as the 1st day of treatment. It continued for a total of 21 days. Body weight and blood glucose levels were monitored on days 0, 3, 7, 14, and 21 of post-treatment. Diabetes was confirmed by evaluating the blood glucose levels in all the rats prior to STZ administration. On the 3rd day, after 72 hours, the blood glucose levels were evaluated, and rats with blood glucose levels exceeding 250 mg/dl were considered diabetic and included in the study [33].

Collection of blood sample and blood glucose determination

Blood samples were collected from the retro-orbital vein of the rats during the study. Fasting blood glucose levels were measured on day 0, 3, 7, 14, and 21 using the Accu-Check Active glucometer. The blood glucose levels were expressed in mg/dl. This method of blood glucose estimation offered sufficient sensitivity and had the advantage of requiring only a small amount of blood (1-2 µl) for analysis. The blood samples were collected from the retro-orbital vein and placed on the glucose test strip of the glucometer.

Statistical analysis

The data were presented as the mean ± SEM. One-way analysis of variance (ANOVA) was used to assess the statistical significance among the groups, followed by post-hoc analysis using Dunnett's t-test. P values less than 0.05 were considered significant.

Results

After each successive Soxhlation extraction, the crude extracts were concentrated by completely evaporating the solvents to obtain the final extraction yield. The leaves of C. paniculatus yielded extracts of 0.475% with petroleum ether and 5.843% with methanol. Table 1 presents the qualitative phytochemical analysis results of the raw leaves of C. paniculatus. The methanolic extract showed a total phenolic content (TPC) of 32.33 mg/gm and total flavonoid content (TFC) of 11.66 mg/gm, as shown in Table 2 and Figure 1, 2. The antioxidant activity of the samples was evaluated using the DPPH and ABTS radical cation decolorization assays. Ascorbic acid was used as the standard, and the concentrations ranged from 20µg/ml to 100µg/ml. The scavenging activity of the extracts and standard on the DPPH radical was expressed as the IC50 value, which was 51.70 for the methanolic extract and 22.54 for ascorbic acid, as shown in Table 3. The IC50 value of the methanolic extract was effective and comparable to ascorbic acid, a well-known antioxidant. Similarly, the scavenging activity of the extracts and standard on the ABTS radical was evaluated, and the IC50 value was 40.97 for the methanolic extract and 22.54 for ascorbic acid, as shown in Table 4. The IC50 value of the methanolic extract was effective and comparable to ascorbic acid. The effects of the extract on body weight in diabetic rats are presented in Table 5. Prior to extract administration, all groups showed no significant difference in body weight compared to the normal control group. However, all doses of the extract and the standard showed a significant improvement in body weight at the 3rd, 7th, 14th, and 21st day compared to the diabetic control group. In contrast, the body weight of the diabetic control group was significantly decreased at the 3rd, 7th, 14th, and 21st day compared to the normal control group. All the groups of animals were affected by diabetes, as indicated by slight changes in blood glucose levels compared to the normal control group. All doses of the extract and the standard showed a significant decrease in blood glucose levels at the 3rd, 7th, 14th, and 21st day compared to the diabetic control group. Conversely, the blood glucose level of the diabetic control group was significantly increased at the 3rd, 7th, 14th, and 21st day compared to the normal control group, as shown in Table 6.

Table 1: Results of extract phytochemical screening of *C. paniculatus*

S. No.	Experiment	Presence or absence of phytochemical	
		Petroleum ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Present	Present
1.2	Mayer's reagent test	Present	Present
1.3	Wagner's reagent test	Present	Present
1.3	Hager's reagent test	Present	Present
2.	**Glycosides**		
2.1	Borntrager test	Absent	Present
2.2	Legal's test	Absent	Present
2.3	Killer-Killiani test	Absent	Present
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Absent
3.2	Fehling's test	Absent	Absent
3.3	Benedict's test	Absent	Absent
3.4	Barfoed's test	Absent	Absent

4.	**Proteins and Amino Acids**		
4.1	Biuret test	Present	Present
5.	**Flavonoids**		
5.1	Alkaline reagent test	Present	Present
5.2	Lead Acetate test	Present	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
7.	**Saponins**		
7.1	Foam test	Present	Present
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Absent	Present
8.2	Libbermann-Burchard's test	Absent	Present

Table 2: Total phenolic and flavonoid content of extracts

Test	**Methanolic extract**
TPC	32.33mg/gm equivalent to Gallic acid
TFC	11.66mg/gm equivalent to Rutin

Table 3: DPPH assay of ascorbic acid and methanolic extract

S. No.	**Conc. (µg/ml)**	**Ascorbic acid (% Inhibition)**	**Methanolic Extract (% Inhibition)**
1.	**20**	50.26853	43.4254
2.	**40**	56.6058	47.0718
3.	**60**	66.058	49.9448
4.	**80**	71.96563	55.2486
5.	**100**	85.49946	65.1934
IC 50 Value		**22.54**	**51.70**

Table 4: ABTS^{+} assay of ascorbic acid and methanolic extract

S. No.	**Conc. (µg/ml)**	**Ascorbic acid (% Inhibition)**	**Methanolic Extract (% Inhibition)**
1.	**20**	50.26853	43.0622
2.	**40**	56.6058	48.80383
3.	**60**	66.058	57.41627
4.	**80**	71.96563	63.51675
5.	**100**	85.49946	70.69378
IC 50 Value		**22.54**	**40.97**

Table 5: Effect of *C. paniculatus* extract on Body weight of the rats

Group	**Treatment**	**Body Weight (gm)**				
		0 day	**3 day**	**7 day**	**14 day**	**21 day**
Group I	Normal Control	212.45±0.200	215.64±0.245	220.45±0.150	221.31±0.315	222.49±0.245
Group II	Diabetic control(STZ)	240.26±0.345	231.19±0.375	225.16±0.452	213.16±0.214	209.14±0.343
Group III	Glibenclamide treated	225.85±0.36**	221.51±0.38**	228.48±0.47**	234.14±0.43**	238.28±0.47**
Group IV	*C. Paniculatus* treated (200 mg/kg bw)	238.15±0.24**	237.25±0.45**	240.26±0.38**	241.41±0.34**	245.15±0.42**
Group V	*C. Paniculatus* treated (400 mg/kg bw)	240.29±0.45**	230.16±0.21**	235.28±0.15**	237.28±0.16**	238.14±0.21**

All values were expressed as mean ± SEM, (n=6 rats),*p<0.05, **p<0.001, Vs control, Statistical significance test for comparison with control followed by one way ANOVA

Table 6: Effect of test samples of extract on blood glucose level in experimental rats

Groups	Treatment	Blood glucose level (mg/dl)				
		0 day	3 day	7 day	14 day	21 day
Group I	Normal Control	110.12±0.50	107.25±0.46	115.36±0.25	102.45±0.14	105.14±0.25
Group II	Diabetic control (STZ)	215.45±0.46	243.14±0.52	293.14±0.34	284.14±0.45	295.14±0.24
Group III	Glibenclamide treated	201.45±0.76**	199.25±0.64**	175.39±0.75**	152.48±0.15**	145.12±0.46**
Group IV	*C. Paniculatus* treated (200 mg/kg bw)	205.14±0.48**	198.14±0.46**	184.25±0.35**	173.14±0.49**	169.45±0.18**
Group V	*C. Paniculatus* treated (400 mg/kg bw)	201.48±0.42**	200.43±0.45**	180.24±0.34**	174.14±0.35**	162.85±0.48**

All values were expressed as mean ±SEM, (n=6 rats), *p<0.05, **p<0.001, Vs control, Statistical significance test for comparison with control followed by one way ANOVA

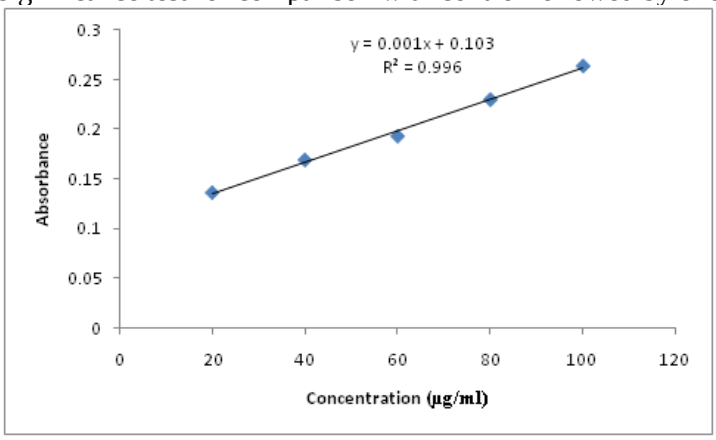

Figure 1: Graph of estimation of total phenolic content (Gallic acid)

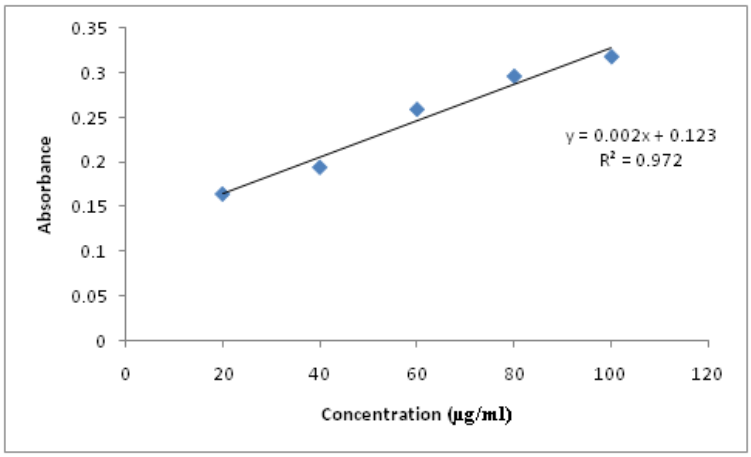

Figure 2: Graph of estimation of total flavonoid content (Rutin)

Discussions

The process used for phytochemical analysis is simple, rapid, and cost-effective, providing immediate results for the various phytochemical constituents present in a mixture. Table 1 displays the results of a qualitative phytochemical analysis of untreated C. paniculatus leaves. The antioxidant activity is determined by the reduction of the $DPPH^{\cdot}$ radical in the test sample. $DPPH^{\cdot}$ accepts an electron or hydrogen radical, resulting in the formation of a stable diamagnetic molecule. The color change from purple to yellow indicates the reducing ability of antioxidants towards the DPPH stable free radical. The reduction capacity of the DPPH radical is assessed by the decrease in absorbance at 517 nm, and the methanol extracts of leaves showed a percentage inhibition of 65.20%. Ascorbic acid was used as a standard antioxidant and exhibited 85.50% inhibition. The ABTS assay is a simple and indirect method for evaluating the antioxidant activity of natural compounds. The decolorization of ABTS is used to measure the reduction of the radical cation, expressed as the percentage inhibition of absorbance at 734 nm. The methanol extracts of leaves exhibited maximum

inhibition at 100 µg/ml, with a percentage of 70.70%. Ascorbic acid was used as a standard antioxidant with 85.50% inhibition.

STZ is a toxic glycoside produced by the gram-positive bacterium Streptomyces achromogenes. It accumulates in pancreatic cells through the glucose transporter 2 (GLUT2), leading to decreased expression. The alkylating activity of STZ alters biological macromolecules, disrupts DNA, and induces cell death, resulting in insulin-dependent diabetes. The diabetic control group experienced a significant decrease in body weight, likely due to muscle wasting and loss of tissue proteins. In the current study, the treatment groups, including glibenclamide and methanolic extracts of C. paniculatus, showed a significant prevention of muscle loss caused by hyperglycemia, as evidenced by the preservation of body weight. This may be attributed to increased plasma insulin levels or improved blood glucose transport in peripheral tissues. The methanolic extracts of C. paniculatus were found to increase plasma insulin levels and exhibit potential anti-diabetic effects.

Conclusion

Medicinal plants are commonly utilized for the discovery and evaluation of phytochemical constituents that hold potential for the development of novel medications. The findings of the present study are consistent with previous investigations regarding the phytochemical composition of C. paniculatus. The presence of these phytochemical elements is of great interest due to their potential antioxidant activity, which is often associated with therapeutic benefits in the treatment of cardiovascular disorders.

The anti-hyperglycemic properties of the C. paniculatus leaf extract demonstrated in this study are comparable to those of the standard medication employed. This could be attributed to the extract's ability to enhance insulin production from the regenerated pancreatic beta cells and inhibit α-amylase, an enzyme involved in carbohydrate metabolism. The results of this research further support the traditional use of this plant in the management of diabetes.

References

1. Shihabudeen MS, Priscilla HHD, Thirumurugan K, Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants, International Journal of Pharma Sciences and Research, 1, 2010, 430-434.
2. Cook N. Flavonoids--Chemistry, metabolism, cardioprotective effects, and dietary sources. J Nutr Biochem. 1996; 7(2):66–76.
3. Kumpulainen JT, Salonen JT. Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease 1999.
4. Tanaka T, Kusano R, Kouno I. Synthesis and antioxidant activity of novel amphipathic derivatives of tea polyphenol. Bioorg Med Chem Lett 1998; 8(14):1801–6.
5. Young IS. Antioxidants in health and disease. J Clin Pathol 2001; 54(3):176–86.
6. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet 1994; 344(8924):721–4.
7. Halliwell B. Biochemistry of oxidative stress: Figure 1. Biochem Soc Trans 2007; 35(5):1147–50.
8. Singh M and Naithani M. Phytochemical estimation and antioxidant activity of seed extract of millets traditionally consumed by common people of Uttrakhand, India. Int.J .Biol .Pharm .Allied Sci... 2014; 3(10): 2389-400.
9. Litescu SC, Sandra AV Eremia, SAV, Diaconu M, Tache A and RaduG.L. Biosensors Applications on Assessment of Reactive Oxygen Species and Antioxidants. In: Vernon Somerset, editor. Environmental Biosensors.In tech open 2011,p 95-114
10. Ramadan-Hassanien MF. Total antioxidant potential of juices, beverages and hot drinks consumed in Egypt screened by DPPH in vitro assay. Grasas y Aceites 2008; 59(3):254–9.
11. Suhaj M. Spice antioxidants isolation and their antiradical activity: a review. J Food Compos Anal. 2006; 19(6–7):531–7.
12. Eseyin O, Ebong P, Eyong E, Awofisayo O, Agboke A. Effect of Telfairia occidentalis on oral glucose tolerance in rats. Afr J Pharm Pharmacol 2010; 4: 368-372.
13. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed 2012; 2: 320-330.

14. Rajan M, Kumar VK, Kumar PS, Swathi KR, Haritha S. Antidiabetic, antihyperlipidaemic and hepatoprotective activity of methanolic extract of Ruellia tuberose Linn. leaves in normal and alloxan induced diabetes. J Chem Pharm Res 2012; 4: 2860-2868.
15. Rajasekar V, Kirbanandan S, Lakshmi BS. Assessment of antidiabetic activity of Syzygium jambolanum using in vitro model. Am J Infect Dis 2010; 5: 99-105.
16. Malviya N, Jain S, Malviya S. Antidiabetic potential of medicinal plants. Acta Pol Pharm 2010; 67: 113-118.
17. Dangi KS, Mishra SN. Antihyperglycemic, antioxidant and hypolipidemic effect of Capparis aphylla stem extracts in streptozotocin induced diabetic rats. Biol Med 2010; 2: 35-44.
18. Yalniz M, Pour P M (2006) Toxicology of the endocrine pancreas. Taylor and Francis group, Boca Raton, Florida, USA, 542-545.
19. Ghasemi A, Khalifi S, Jedi S (2014) Streptozotocin-nicotinamide-induced rat model of type 2 diabetes (review). Acta Physiol Hung 101: 408-420.
20. Nayak Y, Hillemane V, Daroji VK, Jayashree BS, Unnikrishnan MK (2014) Antidiabetic activity of benzopyrone analogues in nicotinamide-streptozotocin induced type 2 diabetes in rats. The Scientific World Journal.
21. Eleazu CO, Eleazu KC, Chukwuma S, Essien UN (2013) Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. J Diabetes Metab Disord 12: 60.
22. Sharada M, Ahuja A, Kaul MK (2003) Regeneration of plantlets via callus cultures in Celastrus paniculatus Willd-A rare endangered medicinal plant. J Plant Bio Chem Biotech 12(1):65–69.
23. Khanna C (2007) Conservation of some useful medicinal plants of Haridwar district in Uttaranchal state. Medicinal Plants: Conservation and Cultivation, Uttaranchal, pp 147–166
24. Mude N, Ingle A, Gade A, Rai M (2009) Synthesis of silver nanoparticles using callus extract of Carica papaya—a first report. J Plant Biochem Bio Tech 18(1):83–86.
25. Jain DK, Gupta S, Jain R, Jain N. Anti-inflammatory Activity of 80% Ethanolic Extract of Acorus calamus Linn. Leaves in Albino Rats. Research J. Pharm. and Tech 2010; 3 (3): 882-884.
26. Dutta R, Sharma MK, Khan A, Jha M. Phytochemical and in vitro antioxidant assay of Fumaria officinalis leaf extract. Journal of Advanced Scientific Research. 2020 Aug 10; 11(03):176-82.
27. Gadekar S, Goyal S, Khan A, Jha M. Chemopreventive action of sphaeranthus indicus on dmba-induced skin papillomagenesis in mice. Journal of Advanced Scientific Research. 2020; 11(3):161-167.
28. Pradhan A, Jain P, Pal M, Chauhan M, Jain DK. Qualitative and quantitative determination of phytochemical contents of hydroalcoholic extract of salmalia malabarica. Pharmacologyonline 2019; 1:21-26.
29. Joshi S, Parkhe G, Aqueel N, Dixit N, Jain DK. Estimation of total phenolic, total flavonoids and total protein content of hydroalcoholic extract of Anacyclus pyrethrum. Pharmacologyonline 2019; 1:27-33.
30. Gulçin I, Elias R, Gepdiremen A, Boyer L. Antioxidant activity of lignans from fringe tree (Chionanthus virginicus L.). Eur Food Res Technol 2006; 223: 759-767.
31. Zheleva-Dimitrova D, Nedialkov P, Kitanov G. Radical scavenging and antioxidant activities of methanolic extracts from Hypericum species growing in Bulgaria. Pharmacogn Mag. 2010 Apr;6(22):74–8.
32. Etuk EU. Animals models for studying diabetes mellitus. Agric Biol JN Am. 2010;1(2):130–4.
33. Kumar S, Kumar V, Prakash OM. Antidiabetic and hypolipidemic activities of Kigelia pinnata flowers extract in streptozotocin induced diabetic rats. Asian Pac J Trop Biomed. 2012;2(7):543–6.
34. Jag Mohan. Organic spectroscopy principles and applications, Narosa publishing House, Daryagani, Delhi, 2005, 2Edn.
35. Halliwell B, Gutteridge JM, Aruoma OI. The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals, Anal Bio chem. 1987; 165:215-9.
36. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 2001; 50:536-46.
37. Cheng D, Liang B, Li Y. Antihyperglycemic effect of *Ginkgo biloba* extract in streptozotocin-induced diabetes in rats. Biomed Res Int 2013; 2013:162724.
38. Wilcox G. Insulin and Insulin Resistance. Clin Biochem Rev 2005; 26:19-39.