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Original Research Article

EXTRACTION, PHYTOCHEMICAL ANALYSIS AND ANTIDIABETIC ACTIVITY OF *LITSEA GLUTINOSA*

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ABSTRACT

Diabetes is anticipated to affect 438 million people (7.8% of the adult population) by 2030, according to recent estimates. The current medications for diabetes have a number of undesirable side effects. The *Litsea glutinosa* plant has numerous therapeutic characteristics and is traditionally used for a variety of alignments. The aim of this study is analyzing anti diabetic effect of *Litsea glutinosa*. The plant material was collected & subjected to hydroalcohlolic extraction followed by qualitative & quantitative analysis. The In vivo antidiabetic study was then carried out in STZ induced diabetic rats. The hydroalcoholic bark extract of *Litsea glutinosa* was given to particular group of rats in 100mg/kg & 200mg/kg of concentration. Glibenclamide was used as standard. Results revealed that Phytochemical screening revealed presence of Alkaloids, Carbohydrates, Glycosides, Saponins, Phenols, Flavonoids, Proteins. Total alkaloid & phenol content was found to be 0.954 mg/100mg 0.417 mg/100mg. The blood glucose level in in 100 mg/kg & 200mg/kg on 21st day was found to be 214.1±1.52 & 170.8±1.66 mg/dl. While in case of Glibenclamide it was observed to be 150.3±1.85mg/dl. The total cholesterol level in 100 mg/kg & 200mg/kg extract treated rats was observed to be 124.0 ± 1.50 & 109.1 ± 1.30 mg/dL respectively. For Glibenclamide, treated rats total cholesterol content in blood was noted to be 101.4 ± 1.10. Further the level of triglyceride in 100 mg/kg & 200mg/kg extract treated rats was seen to be 124.00 ± 1.80 &114.00 ± 1.80 mg/dL respectively. The protein content in 200 mg/kg extract group was observed to be 7.43 ± 0.60 g/dL. The final weight of rats in hydroalcoholic bark extract of *Litsea glutinosa* (200 mg/kg) was observed to be 185.00 ± 1.40. In summary, the blood glucose levels seen after treating diabetes-induced rats with hydroalcoholic extract of *Listea glutinosa* were equivalent to those obtained after treatment with glibenclamide.

Keywords: Diabetes, Medicinal plants, Phytochemicals, Listea glutinosa, STZ induced diabetes, Glibenclamide

INTRODUCTION

Diabetes mellitus (DM), usually known as just diabetes, is a set of metabolic illnesses characterised by persistently elevated blood sugar levels. This elevated blood sugar level causes symptoms such as frequent urination, thirst, and hunger. Diabetes, if left untreated, can lead to a slew of consequences. Diabetic ketoacidosis and nonketotic hyperosmolar coma are examples of acute complications. India is the world's second most populous country, with more people suffering from type 2 diabetes than any other country, as the

disease affects all genders and all age groups (Asmat *et al*., 2016; Kiadaliri *et al*., 2013).

Diabetes is anticipated to affect 438 million people (7.8% of the adult population) by 2030, according to recent estimates. Some factors, such as stress, fast urbanisation, and a significant increase in purchasing power, lifestyle convenience, and metro life, have contributed to health difficulties and an increase in the number of persons suffering from these diseases. Diabetes and its complications cost more than $100 billion per year, and problems are significantly less prevalent and severe in those with well-controlled blood sugar levels (Harding *et al*., 2019; Khan *et al*., 2020).

There are currently various antidiabetic medications on the market to treat hyperglycemia, most of which work by improving insulin sensitivity, supplementing insulin, increasing insulin secretion, and stimulating glucose absorption. However, both metformin and sulfonylureas are associated with a number of undesirable side effects, including diarrhoea and lactic acidosis (shown by metformin) and hepatic failure, weight gain, tachycardia, and hypothyroidism (shown by sulfonylureas)(Harding *et al*., 2019; Khan *et al*., 2020).

Because the adverse effects of drug treatment are not always satisfactory in maintaining normal levels of blood glucose, many medicinal plants have been identified as a potential source of antidiabetic principles that are widely used for the treatment of diabetes mellitus in various traditional systems of medicine around the world, and many of them are known to be effective against diabetes (Mahabir and Gulliford, 1997).

The plant *Litsea glutinosa* is widely grown in tropical and subtropical climates worldwide, including India, Japan, Taiwan, and many parts of China. The *Litsea glutinosa* plant has numerous therapeutic characteristics and is traditionally used for a variety of gastrointestinal disorders and diseases such as abdominal discomfort, indigestion, diarrhoea, gastroenteritis and diabetes, edoema, traumatic injuries, colds, arthritis, and asthma. The Litsea plant is well recognised for its essential oil, which has antibacterial properties against a wide range of microorganisms. This plant possesses antioxidant and anti-parasitic characteristics, as well as the ability to eradicate acute and genetic toxicity and cytotoxicity, which aids in the prevention of many cancers (Jamaddar *et al*., 2022). The aim of this study is analyzing anti diabetic effect of *Litsea glutinosa*.

MATERIALS AND METHODS

Chemical and reagent

Potassium Mercuric Iodide, Potassium Iodide, Iodine, Ferric chloride, Lead acetate, Nitric acid, Copper acetate, Aluminum chloride Potassium Bismuth Iodide, Picric acid, Sodium nitropruside and Sodium hydroxide obtained from Loba Chemical Pvt Ltd (Mumbai, India). Hydrochloric acid, methanol and ethanol were obtained from Merck Ltd, Mumbai, India. Atropine and Gallic acid were purchased from Hi Media, Mumbai. All solvents and reagents were of analytical grade.

Collection of plant material

The collection of plant material is an essential step in various scientific, agricultural, and horticultural endeavors. It involves the careful

gathering and preservation of plant specimens for further study, experimentation, propagation, or conservation purposes. The method of collecting plant material depends on the specific objectives, the type of plant, and the desired outcome. Barks of *Litsea glutinosa* were collected from local area of Bhopal.

Extraction procedure

Defatting of plant material

Barks of *Litsea glutinosa* were shade dried at room temperature. 36 gram of dried bark was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

The maceration process is a method used to extract the active compounds from plant materials, such as herbs, roots, or flowers, by soaking them in a liquid solvent. This technique has been employed for centuries in various traditional medicine systems and is still widely used today in herbal preparations and the production of natural remedies, tinctures, and extracts.

The maceration process is just one of several extraction methods available, and its suitability depends on the specific plant material and desired compounds. Different plants may require different solvents and extraction techniques to achieve optimal results. Defatted bark of *Litsea glutinosa* were extracted with hydroalcoholic solvent (Ethanol: Water: 80:20) using maceration process (24hrs). The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts (Casassa and Harbertson, 2014).

Estimation of total alkaloids content

The plant extracts (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract (Ncube *et al*., 2015).

Estimation of total phenol content

The total phenolic content was estimated according to the FC method. The aliquots of the extract was taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5ml of sodium carbonate solution (20%) were added. After mixing, solution was incubated & the absorbance was recorded at 765nm against the reagent blank. Using galllic acid standard curve was prepared. Using the standard curve, the total phenolic content was calculated and expressed as gallic acid equivalent in µg/mg of extract.

In -vivo antidiabetic activity of hydroalcoholic bark extract of *Litsea glutinosa* in STZ-induced rats

Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute toxicity

Toxicity studies were carried out in accordance with OECD guidelines, acute oral toxicity study of *hydroalcoholic bark extract of Litsea glutinosa* (OECD; 2000). The *hydroalcoholic bark extract of Litsea glutinosa* (50, 100, 150, 200, 300 mg/kg/day) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under examination for mortality as well as any behavioral changes.

Induction of Experimental Diabetes in Rats

Sterptozotocin (STZ) was dissolved in ice cold 0.1M citrate buffer (pH 4.5) was dissolved in normal physiological saline. The animals were allowed to drink 5% glucose solution overnight to overcome STZ induced hyperglycaemia. Non-insulin dependent diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of 60 mg/kg sterptozotocin. The animals were considered as diabetic, if their blood glucose value above 200 mg/dl on 3rd day of STZ injection. Only rats confirmed to have permanent NIDDM (noninsulin-dependent diabetes mellitus) were used for the antidiabetic study. The rats were divided into eight groups (n=6) each randomly, All the test standard substances are administered for 21 days. Blood samples were collected by retro-orbital puncture at 0, 7, 14 and 21 days at the glucose levels were estimated by Glucometer.

Experimental Protocol

Animals were divided into eight groups of 6 rats each

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

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

Group III: Rats (diabetic) were administered *hydroalcoholic bark extract of Litsea glutinosa* (100 mg/kg p.o.)

Group IV: Rats (diabetic) were administered *hydroalcoholic bark extract of Litsea glutinosa* (200 mg/kg p.o.)

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

Table 1: Qualitative chemical tests of extract of *Litsea glutinosa*

S. No.	Bioactive constituents	Test	Hydroalcoholic extract
1	Alkaloids	Hager's Test	+ve
2	Carbohydrates	Fehling's Test	+ve
3	Glycosides	Legal's Test	-ve
4	Saponins	Froth Test	+ve
5	Phenols	Ferric Chloride Test	+ve
6	Flavonoids	Lead acetate Test	-ve
7	Proteins	Xanthoproteic Test	+ve
8	Diterpenes	Copper acetate Test	-ve

+ ve – Present, - ve – Absent

Table 2: Estimation of total alkaloids and phenol content in *Litsea glutinosa*

S. No.	Total alkaloids content	Total phenol content
1.	0.954 mg/100mg	0.417 mg/100mg

Table 3: Effect of hydroalcoholic bark extract of *Litsea glutinosa* treatment on blood glucose (mg/dl) in normal and diabetic rats

Group	Treatment	Blood glucose (mg/dl)			
		Days 0	Days 7	Days 14	Days 21
I	Normal	84.1±0.5	91.0±0.65	92.5±0.70	92.7±0.70
II	Diabetic Control (STZ-60 mg/kg)	281.0±1.45	288.9±1.35#	293.4±1.87#	299.65±1.77#
III	Diabetic + hydroalcoholic bark extract of *Litsea glutinosa* (100 mg/kg)	281.25±1.445	284.2±1.500*	231.2±1.52*	214.1±1.52*
IV	Diabetic + hydroalcoholic bark extract of *Litsea glutinosa* (200 mg/kg)	282.6±1.561	218.9±1.525**	189.8±1.76***	170.8±1.66***
V	Diabetic + Glibenclamide (600µg/kg)	280.5±1.9	214.5±1.73***	170.3±1.95***	150.3±1.85***

Values are expressed as mean±S.E.M (n = 6).Values are statistically significant at # p<0.001 vs. normal group; *P < 0.001, **P < 0.01vs. diabetic control group (Two-way ANOVA test).

Table 4: Effect of *hydroalcoholic bark extract of Litsea glutinosa* treatment on biochemical parameters (Triglyceride, Total protein, Total cholesterol) in normal and diabetic rats

Group	Treatment	TG (mg/dL)	Total protein(g/dl)	TC (mg/dL)
I	Normal	80.00 ± 1.50	7.50 ± 0.45	100.00 ± 1.20
II	Diabetic Control (STZ-60 mg/kg)	200.0 ± 2.10#	5.30 ± 0.53	231.0 ± 2.50#
III	Diabetic + hydroalcoholic bark extract of *Litsea glutinosa* (100 mg/kg)	124.00 ± 1.80**	7.32± 0.50**	124.0 ± 1.50**
IV	Diabetic + hydroalcoholic bark extract of *Litsea glutinosa* (200 mg/kg)	114.00 ± 1.80***	7.43 ± 0.60***	109.1 ± 1.30***
V	Diabetic + Glibenclamide (600µg/kg)	100.02±1.60***	7.44 ± 0.50***	101.4 ± 1.10***

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

Table 5: Effects of hydroalcoholic bark extract of *Litsea glutinosa* on body weight

Group	Treatment	Initial weight (g)	Final weight (g)
I	Normal	160.00 ± 2.00	180.10 ± 1.50
II	Diabetic Control (STZ-60 mg/kg)	190.00 ± 1.40	150.00 ± 1.70#
III	Diabetic + hydroalcoholic bark extract of *Litsea glutinosa* (100 mg/kg)	194.00 ± 1.30	187.00 ± 1.30**
IV	Diabetic + hydroalcoholic bark extract of *Litsea glutinosa* (200 mg/kg)	196.00 ± 1.50	185.00 ± 1.40***
V	Diabetic + Glibenclamide (600µg/kg)	195.00 ± 1.50	180.00 ± 1.40***

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

RESULTS AND DISCUSSION

Phytochemical screening revealed presence of Alkaloids, Carbohydrates, Glycosides, Saponins, Phenols, Flavonoids, Proteins. This plant extract's hypoglycemic effect could be due to the presence of phytochemical elements such as flavonoids, free and bound anthraquinones, tannins, terpenoids, sterols, saponins, and alkaloids, which have been linked to anti-diabetic action. flavoonoids boost lipogenesis and glucose metabolism As a result, blood sugar levels are reduced. The alkaloids induce pancreatic islet regeneration, so restoring Secretion of insuln. Tannins and saponins have also been linked to hypoglycemic action. Terpenoids in the plants are heart-friendly because they aid to lower diastolic blood pressure and blood sugar levels. Anthraquinones, which have previously been shown to reduce blood glucose levels, are also utilised in the treatment of peripheral neuropathy.

Total alkaloid & phenol content was found to be 0.954 mg/100mg 0.417 mg/100mg. The blood glucose level in in 100 mg/kg & 200mg/kg on 21st day was found to be 214.1±1.52 & 170.8±1.66 mg/dl. While in case of Glibenclamide it was observed to be 150.3±1.85mg/dl.

The total cholesterol level in 100 mg/kg & 200mg/kg extract treated rats was observed to be 124.0 ± 1.50 & 109.1 ± 1.30 mg/dL respectively. For Glibenclamide, treated rats total cholesterol content in blood was noted to be 101.4 ± 1.10.

Further the level of triglyceride in 100 mg/kg & 200mg/kg extract treated rats was seen to be 124.00 ± 1.80 &114.00 ± 1.80 mg/dL respectively. The decrease in blood TG level is significant since recent research reveal that TG is independently connected to coronary heart disease.

The protein content in 200 mg/kg extract group was observed to be 7.43 ± 0.60 g/dL. The final weight of rats in hydroalcoholic bark extract of *Litsea glutinosa* (200 mg/kg) was observed to be 185.00 ± 1.40.

Thus, when compared to low dosage administration, higher doses of *Listea glutinosa* resulted in greater reductions in parameter values. The blood glucose levels seen after treating diabetes-induced rats with hydroalcoholic extract of *Listea glutinosa* were equivalent to those obtained after treatment with glibenclamide. In STZ-induced diabetic rats given with an hydroalcoholic extract of *Listea glutinosa* blood glucose levels, total cholesterol, triglycerides were reduced in a dose-dependent manner.

CONCLUSION

In conclusion, it appears that the hydroalcoholic extract of *L. glutinosa* can reduce hyperglycemia induced by diabetes and attenuate renal, hepatic, and pancreatic problems due to antioxidant components such as linolenic acid, phytol, and neofitadine. This extract can also help to avoid anaemia and blood problems caused by diabetes by managing haematological parameters. These findings can be used to develop an appropriate food supplement to alleviate diabetes issues in the future.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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