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STUDY OF PHYTOCHEMICALS AND *STZ INDUCED* ANTIDIABETIC ACTIVITY OF *LITSEA GLUTINOSA*

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Abstract

Based on recent estimates, 438 million adults (7.8% of the adult population) are expected to have diabetes by 2030. The diabetes drugs that are currently available have a lot of unfavourable side effects. The plant *Litsea glutinosa* is used traditionally for many alignments and has several medicinal properties. This study aims to analyse *Litsea glutinosa*'s anti-diabetic effect. Following the collection of the plant material, it underwent both qualitative and quantitative analysis and hydroalcoholic extraction. Next, using diabetes rats caused by STZ, the in vivo antidiabetic investigation was conducted. The hydroalcoholic bark extract of *Litsea glutinosa* was administered to certain group of rats in 100mg/kg & 200mg/kg of concentration. Standard protocol was to utilise glibenclamide. The presence of alkaloids, carbohydrates, glycosides, saponins, phenols, flavonoids, and proteins was detected by phytochemical screening, according to the results. It was discovered that the total concentration of phenol and alkaloids was 0.417 and 0.954 mg/100 mg. The blood glucose levels measured on the 21st day were 214.1±1.52 & 170.8±1.66 mg/dl in 100 mg/kg & 200 mg/kg, respectively. Glibenclamide, on the other hand, showed an observation of 150.3±1.85 mg/dl. Rats given 100 mg/kg and 200 mg/kg extract had total cholesterol levels of 124.0 ± 1.50 & 109.1 ± 1.30 mg/dL, respectively. The total cholesterol concentration in the blood of rats treated with Glibenclamide was found to be 101.4 ± 1.10. Additionally, the rats given 100 mg/kg and 200 mg/kg extract were found to have triglyceride levels of 124.00 ± 1.80 & 114.00 ± 1.80 mg/dL, respectively. In the 200 mg/kg extract group, the observed protein content was 7.43 ± 0.60 g/dL. Rats in a 200 mg/kg hydroalcoholic bark extract of *Litsea glutinosa* were found to weigh 185.00 ± 1.40 in the end. In conclusion, the blood glucose levels seen following the administration of *Listea glutinosa* hydroalcoholic extract to rats induced with diabetes were comparable to those observed following glibenclamide treatment.

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

Introduction

Diabetes mellitus (DM), sometimes referred to as just diabetes, is a group of metabolic diseases marked by blood sugar levels that remain elevated over time. Frequent urination, thirst, and hunger are indications of a high blood sugar level. If diabetes is not treated, there might be a lot of negative effects. Acute consequences include diabetic ketoacidosis and nonketotic hyperosmolar coma. With the second-highest population in the world, India has the highest rate of type 2 diabetes patients worldwide, affecting people of all ages and genders (Asmat *et al*., 2016; Kiadaliri *et al*., 2013).

Based on recent estimates, 438 million adults (7.8% of the adult population) are expected to have diabetes by 2030. Stress, rapid urbanisation, a notable rise in spending power, ease of living, and living in a metropolis are some of the reasons that have led to health issues and an increase in the number of people afflicted with these diseases. The annual cost of diabetes and its complications is over $100 billion, whereas individuals with well-controlled blood sugar levels experience far fewer and milder effects (Harding *et al*., 2019; Khan *et al*., 2020).

To treat hyperglycemia, there are currently a number of antidiabetic drugs available; the majority of these drugs function by enhancing insulin sensitivity, supplying extra insulin, raising insulin production, and promoting the absorption of glucose. Nevertheless, a variety of negative side effects are linked to both metformin and sulfonylureas, such as lactic acidosis and diarrhoea (exhibited by metformin) and hepatic failure, weight gain, tachycardia, and hypothyroidism (exhibited by sulfonylureas) (Harding *et al*., 2019; Khan *et al*., 2020).

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 medical systems around the world, and many of them are known to be effective against diabetes, because the side effects of drug treatment are not always satisfactory in maintaining normal levels of blood glucose (Mahabir and Gulliford, 1997).

Around the world, including India, Japan, Taiwan, and many regions of China, the plant *Litsea glutinosa* is commonly grown in tropical and subtropical climates. The *Litsea glutinosa* plant has several medicinal qualities and is traditionally used for a number of

gastrointestinal illnesses and diseases such as abdominal discomfort, indigestion, diarrhoea, gastroenteritis and diabetes, edoema, traumatic injuries, colds, arthritis, and asthma. The essential oil of the Litsea plant is widely known for having antibacterial qualities against a variety of microbes. This plant can prevent many malignancies because it has anti-oxidant and anti-parasitic properties. It can also eliminate cytotoxicity and acute and genetic toxicity (Jamaddar et al., 2022). This study aims to analyse *Litsea glutinosa's* anti-diabetic effect.

Materials & Methods

Collection of plant material

A crucial first step in many horticultural, scientific, and agricultural endeavours is gathering plant material. In order to further research, experimentation, propagation, or conservation, it entails the meticulous collection and preservation of plant specimens. The exact goals, the kind of plant, and the intended result determine the best way to gather plant material. *Litsea glutinosa* bark was gathered from the surrounding Bhopal area.

Extraction procedure

Defatting of plant material

The bark of *Litsea glutinosa* was allowed to air dry in the shade. A thirty-six-gram sample of dried bark was ground into a coarse powder and macerated in petroleum ether to extract the contents. Until the material had undergone defatting, the extraction process was maintained. **Extraction by maceration process**

By immersing plant materials—such as flowers, roots, or herbs—in a liquid solvent, the maceration procedure extracts the active ingredients. This method has been utilised for millennia in many ancient medical systems and is still commonly applied today in the manufacturing of tinctures, extracts, and herbal medications.

The appropriateness of each extraction technique, including the maceration process, varies depending on the particular plant material and targeted chemicals. For best results, different plants might need different extraction methods and solvents. Using a maceration procedure lasting 24 hours, defatted bark of *Litsea glutinosa* was extracted

using a hydroalcoholic solvent (ethanol: water: 80:20). Above the points at which they boiled, the extract evaporated. Ultimately, the dried extracts' % yields were computed (Casassa and Harbertson, 2014).

Estimation of total alkaloids content

1 milligramme of the plant extract was diluted in 1 millilitre of 2 N HCl, then filtered. After this solution was moved to a separating funnel, five millilitres each of phosphate buffer and bromocresol green solution were added. The mixture was collected in a 10-ml volumetric flask and diluted to the volume with chloroform after being vigorously agitated with 1, 2, 3, and 4 ml of the chloroform. In the same way as previously mentioned, a series of reference standard solutions containing 40, 60, 80, 100, and 120 µg/ml of atropine were created. Using a UV/Visible spectrophotometer, the absorbance of the test and standard solutions was measured at 470 nm in relation to the reagent blank. The measure of total alkaloid concentration was mg of AE/100 mg of extract (Ncube *et al*., 2015).

Estimation of total phenol content

The FC technique was used to estimate the total phenolic content. An aliquot of the extract was placed in a test tube and filled with distilled water to make a volume of one millilitre. Next, 2.5 millilitres of sodium carbonate solution (20%) and 0.5 millilitres of Folin-Ciocalteu reagent (1:1 with water) were added. Following mixing, the solution was incubated, and the absorbance at 765 nm was measured in comparison to the reagent blank. A standard curve using galllic acid was created. The total phenolic content was determined using the standard curve and reported as the 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 weighing between 150 and 200 g were kept in groups of six in controlled settings with a standard 12-hour light/dark cycle and a temperature and humidity of 25±2 °C. Water was available at all times, along with conventional rat feed. Prior to doing the trials, the rats were given seven days to become used to the lab environment. Every

experiment was conducted from 8:00 to 15:00 in a quiet room. Each series of trials had a different group of six rats. The Ministry of Environment and Forests, Government of India, New Delhi, India, established the Institutional Animal Ethics Committee (IAEC) to oversee and regulate the use of experimental animals. The IAEC granted approval for the animal experiments.

Acute toxicity

The acute oral toxicity study of the hydroalcoholic bark extract of *Litsea glutinosa* was conducted in compliance with OECD recommendations for toxicity studies (OECD, 2000). Six groups of rats (n = 6) were given the hydroalcoholic bark extract of *Litsea glutinosa* orally for four days at doses of 50, 100, 150, 200, and 300 mg/kg/day. The rats were observed for behavioural changes and death.

Induction of Experimental Diabetes in Rats

The 0.1M citrate buffer (pH 4.5) was cooled to a room temperature before being mixed with normal physiological saline to dissolve streptozotocin (STZ). Overnight, the animals were given a 5% glucose solution to help them overcome the hyperglycemia caused by STZ. Rats who were starved for the entire night were given a single intraperitoneal injection of 60 mg/kg streptozotocin to cause non-insulin dependent diabetes mellitus. If an animal's blood glucose level was more than 200 mg/dl on the third day after receiving a STZ injection, it was classified as diabetic. For the antidiabetic trial, only rats with persistent noninsulin-dependent diabetes mellitus (NIDDM) were employed. The test standard drugs are administered to the rats for a period of 21 days after they were randomly assigned to eight groups of six each. At 0, 7, 14 and 21 days, blood samples were taken by retro-orbital puncture, and the Glucometer was used to estimate the glucose levels.

Experimental Protocol

There were eight groups of six rats each for the animals:

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.)

Results & Discussion

Alkaloids, carbohydrates, glycosides, saponins, phenols, flavonoids, and proteins were detected by phytochemical screening. This plant extract's hypoglycemic impact could be attributed to the presence of phytochemical constituents such as flavonoids, free and bound anthraquinones, tannins, terpenoids, sterols, saponins, and alkaloids, which have been associated to anti-diabetic action. Flavonoids increase glucose metabolism and lipogenesis. Blood sugar levels are consequently lowered. The alkaloids promote pancreatic islet regeneration, which reinstates insulin secretion. Hypoglycemic effect has also been associated with tannins and saponins. Because they help to reduce blood sugar and diastolic blood pressure, terpenoids found in plants are heart-healthy. Anthraquinones are also used to treat peripheral neuropathy; they have been demonstrated to lower blood glucose levels.

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.

In the 200 mg/kg extract group, the observed protein content was 7.43 ± 0.60 g/dL. Rats in a 200 mg/kg hydroalcoholic bark extract of *Litsea glutinosa* were found to weigh 185.00 ± 1.40 in the end.

Therefore, higher doses of *Listea glutinosa* led to bigger reductions in parameter values when compared to low dosage administration. After administering *Listea glutinosa* hydroalcoholic extract to diabetic rats, the blood glucose levels observed were comparable to those following glibenclamide treatment. When a hydroalcoholic extract of *Listea glutinosa* was administered to STZ-induced diabetic rats, blood glucose, total cholesterol, and triglycerides were all lowered in a dose-dependent manner.

Conclusion

As a result of antioxidant components such linolenic acid, phytol, and neofitadine, it appears that the hydroalcoholic extract of *L. glutinosa* can attenuate renal, hepatic, and pancreatic issues as well as lower hyperglycemia brought on by diabetes. By controlling haematological factors, this extract can also help prevent anaemia and blood issues brought on by diabetes. These results can be applied to the development of a suitable dietary supplement to address future diabetic problems.

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