Evaluation of Antidiabetic and Antihyperlipidemic Activities of *Chrysanthemum indicum*Flower Extract on Streptozotocin-induced Diabetic Rats

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Abstract

It was the aim of the study to determine the of *Chrysanthemum indicum* phytochemical composition and its ability to reduce the risk of developing type 2 diabetes and hyperlipidemia in rats. The flowers were extracted with ethanol and water as solvents using soxhlet apparatus. The plant extracts were used to determine presence of phytochemicals in them using different tests. Diabetes was induced on animals using streptozotocin (STZ) 65 mg/kg through intra-peritoneal route. Antidabetic activity of animals was evaluated by administering *C. indicum* extract of 200 mg/kg and 400 mg/kg doses via oral route. The phytochemical investigation study revealed presence of alkaloid, glycosides, tannins, carbohydrates and flavonoids. The antidiabetic study of *C. indicum* extract exhibited that blood glucose level of diabetic rats was significantly reduced to 92.11 ±5.71 (200 mg/kg) and 83.38 ±3.99 (400 mg/kg) after treatment with test drug as compared to diabetic control group. Administration of *C. indicum* (400 mg/kg) for 21 days significantly ($p<0.001$) reduced the level of serum total cholesterol (108.64 ±5.94 mg/dL) and triglyceride (85.32 ±4.04 mg/dL) while increasing the HDL cholesterol level (38.76 ±3.04 mg/dL) compared to the diabetic control. In diabetic rats, *C. indicum* reduced blood glucose levels and restored the lipid profile, according to the results of this study. It can be concluded from the study that plant extract may be used in the treatment of type-2 diabetes and hyperlipidemic patients after clinical trials on human subjects.

Keywords: STZ, pancreas, blood glucose, lipid

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Introduction

Hyperglycemia and abnormalities in the metabolism of carbohydrates, lipids, and proteins characterise diabetes mellitus (DM), a chronic metabolic illness. An ageing population, a sedentary lifestyle, and changes in the gut microbiota have all been linked to the development of diabetes

mellitus (DM) (Bonora et al., 2021). In diabetes mellitus, either the pancreatic islet cells of Langerhans secrete insufficient amounts of insulin or insulin resistance causes an increase in blood glucose levels, resulting in diabetes (Cantley and Ashcroft, 2015). However, it has reported that after cancer and heart disease, diabetes is the third most common chronic disease in humans.

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes in adults (those over the age of 30), accounting for around 90% of all cases. Carbohydrate-rich foods can lead to hyperglycemia in patients with T2DM (Kyrou et al., 2020). Insulin resistance, reduced insulin production, hyperinsulinemia, and poor insulin-mediated uptake and consumption of glucose are only a few of the numerous issues linked with T2DM. Today, T2DM is a major public health issue, particularly in developing and developing countries. Around 350 million individuals around the world have diabetes, according to the World Health Organization (WHO, 2021).

T2DM is on the rise, and unless drastic steps are taken to avoid it, it may soon become a pandemic. A wide range of adverse consequences have been linked to the use of synthetic medications. Plant-based treatments tend to have fewer or no adverse effects, which makes them increasingly popular throughout the world's population (Ekor, 2014). Since the dawn of time, scientists have been searching for plant components that have a wide range of biological functions. Finding new anti-diabetic medications in plants is still an appealing prospect due to the presence of numerous natural chemicals such as glycosides, alkaloids, terpenoids and flavonoids and carotenoids that have demonstrated alternative and safe effects on diabetes mellitus in animal studies (Tran et al., 2020). In rural areas, herbal remedies play a vital role because of their ease of availability, lack of adverse effects, and low cost of preparing them. In recent years, a lot of study has been done on natural product chemistry with plants from the Chrysanthemum family. More than 40 flowering perennial herb species of the Chrysanthemum genus have been recognised, with more than 20 of these being found in China (Jiang et al., 2021).

Plants of the family Compositae include *Chrysanthemum indicum*. Anti-diabetic and anti-inflammatory properties have been employed as well as the treatment of eye illness in traditional herbal medicines (Chen et al., 2019; Shao et al., 2020). The Chrysanthemum genus has been proven to have anticancer, antioxidant, anti-inflammatory, and antibacterial activities in modern pharmacological investigations. Research shows that this species is useful in preventing cardiovascular disease and lowering blood cholesterol and fat levels (Li et al., 2019). As a result of the strong medicinal and dietary benefit of *Chrysanthemum indicum*, this study sought to investigate the crude extract's anti-diabetic and anti-hyperlipidemic potential. Secondary metabolites found in the plant extracts were also identified.

Materials and Methods

Extraction of *C. indicum* flowers

The flowers of *C. indicum* were collected from the forest Orissa. It was identified and

authenticated by Dr. Mamata Mohapatra, H.O.D.P.G.Department of Botany, Khalikote University,Berhampur, Odisha. The voucher specimen was deposited in the institute via voucher no. 2021/001.The collected flowers were washed, dried, grounded and extracted with hydroethanolic solvent in the ratio of 50:50 using soxhlet apparatus. The extract was then filtered and dried using rotary evaporator. The yield value of the dried extract was 13.44%. The dried extract was then stored at 4°C.

Phytochemical investigation

Test for glycosides

Keller-Killiani test: Two millilitres of acetic acid, a drop of 5% FeCl3, and conc. H2SO4 were added to 2 millilitres of extract. Indicating the presence of glycosides is reddish brown at the point where the two liquid layers meet, with the upper layer appearing bluish green.

Glycosides test: Small amount of extract was taken and shaken it well with 1 ml water. The NaOH solution was then added to the mixture. Glycosides were detected by the yellow colour that emerged.

Concentrate H2SO4 test: One drop of 5% FeCl3 and one drop of concentrated H2SO4 were added to five millilitres of extract. Glycosides are indicated by the presence of a brown ring.

Molisch's test: Using 2 drops of Molisch's reagent and 2 ml of concentrated H2SO4 in a test tube with a little bend, 1 ml of extract was tested using Molisch's test. The presence of glycosides was shown by the formation of a violet ring at the junction.

Test for Tannins

Ferric chloride test: Adding a few drops of 5% ferric chloride solution to 2 ml of test solution resulted in a ferric chloride test. The presence of hydrolysable tannins was shown by the formation of blue color.

Lead acetate test: A few drops of a 10% lead acetate solution were added to 2 ml of extract. The presence of tannins is indicated by the appearance of a yellow or crimson precipitate.

Acetic acid solution: A few drops of Acetic Acid solution were added to 2 ml of extract. To make red color solution, follow these steps.

Potassium dichromate test: A few drops of Potassium dichromate solution were added to a 2 ml extract of Potassium dichromate solution. The appearance of a red ppt on a computer screen.

Dilute iodine solution: A few drops of weak iodine solution were added to a 2 ml extract of iodine solution. The emergence of a reddish color appeared.

Dilute HNO3: A few drops of Dilute HNO3 solution were added to 2ml of extract. The appearance of a reddish-yellow color.

Test for flavonoids

Add lead acetate solution to a little amount of residue. Precipitation of a yellow color is produced as a result of this. Sodium hydroxide, when added in increasing

amounts, causes the residue to become colored, which fades when acid is added.

Test for carbohydrates

Benedict's test

In order to study carbohydrates, Benedict's reagent was used. An extract of 5mg was mixed with a few drops of benedict's reagent, then heated, and the reddish brown precipitate was detected with the presence of carbohydrate.

Molisch's test

2 mg extract was placed in the test tube, followed by the addition of 1 ml of Molisch's solution. The mixture was thoroughly agitated before being added to the container. 2ml of concentrated Sulphuric acid was carefully put into the test tube after that. Carbohydrate was detected by the appearance of a violet interface ring.

Test for alkaloid

Mayer's test

In the test tube, 2 ml of extract was mixed with a 1% concentration of hydrochloric acid HCl, and the resulting solution was gently warmed to dissolve the extract. Because Potassium mercuric iodine is present in Mayer's reagent, the red color indicates the presence of alkaloids.

Wagner's test

An extract of *C. indicum* was used in this experiment, and 0.5 millilitres of Wagner reagent was added to the solution, which was then thoroughly shaken before being analysed. Alkaloids can be seen in the reddish brown color of the plant. Iodine gives it a reddish brown color and makes it an insoluble complex with the color brown reddish.

Test for saponin

In order to determine the presence of saponin in the aqueous extract, a foam test was carried out using 1 ml of the extract and 5 ml of distilled water. Once the distilled water had been added, it was shook vigorously until foam had formed. The mixture was shaken vigorously with the addition of a few foams and 2 drops of olive oil. Saponin emulsion was created.

Test for steroid

Sulfuric acid and acetic acid solutions were added to a chloroform solution containing 2 mg of *Chrysanthemum indicum* extract. Steroids were found to be the cause of the greenish color. 3 drops of strong sulphuric acid were applied to the 2 mg extract in Salkowski's experiment. Stimulant-induced red coloration is a sign of steroid use.

Experimental protocol

Wistar rats ranging between 175-200 gm were procured from the Central animal house of the Royal College of Pharmacy, Orissa. The study's protocol was approved by the institute's IAEC committee. Our animals were kept in an environment with a constant temperature (25 ± 2°C) and relative humidity (60 ±5%) for one week using a 12-hour light/dark cycle. In polypropylene cages, they were fed a conventional laboratory diet (Lipton India Ltd.) and had unrestricted access to water.

Acute toxicity studies

"Up-and-down" approach was used in healthy adult albino mice of any gender, according to CPCSEA suggested "OECD" guidelines 425 for the acute toxicity investigation. Healthy adult albino mice have to be acclimated in their cages for five days before to experimentation. The animals fasted the night before dosing. It was determined that each animal was weighed and divided into four groups of five during the fasting phase.A stomach tube gave single doses of 55, 175, 550, and 2000 mg/kg of the drug extracts. The animals' behavioural, neurological, and autonomic profiles were carefully scrutinized. At this early stage, preliminary pharmacological investigations were done to evaluate the acute effects and LD50 of the root extracts of *P. granatum* (ethanol and aqueous extract). Even at the maximum dose of 2000mg/kg body weight, no one died. On the other hand, specific autonomic responses increased in size in a dose-dependent manner. There was an increase in irritability and a decrease in pain response.

Evaluation of antidiabetic activity of *C. indicum* flower extract

Experimental animals

There were five groups of six rats; each group containing six rats. Two groups were formed, one of which served as a negative control and the other as a positive control. Group III: Extract 200 mg/kg body weight; Group IV: Extract 400 mg/kg body weight; Group V: Standard medication glibenclamide at 100 mg/kg body weight in the reference group.

Induction of diabetes

Solution of STZ in 0.1 M citrate buffer (50 mg/kg of body weight) in 1 ml/kg intraperitoneal injection was administered. Within two days of receiving STZ injections, the animals develop hyperglycemia. The diagnosis of diabetes was verified by testing the fasting blood glucose level 48 hours following the injection of STZ. Grouping STZ rats with a blood glucose level of more than 200 mg/dl into separate diabetes groups was done for additional research purposes.

Anti-diabetic Activity

To help them become used to the new environment, the rats were housed in a cage for three days before being fasted overnight. STZ was dissolved in normal saline and injected intraperitoneally into rats to test for diabetes. After the STZ treatment, all the animals were given food and drink. Only diabetic rats with a fasting blood glucose level of more than 200 mg/dL were considered to be diabetic and employed in the experiment, which was conducted two days following the injection of STZ. Glucose levels in the blood were measured using blood drawn from the eyes of volunteers. This person's blood sugar level was monitored using a glucometer (one-touch).

Antidyslipidemic activity of extract in streptozotocin-induced diabetic rats

Overdoses of sodium pentobarbitone at a concentration of 150 mg/kg IP were used to kill overnight fasting rats on day 21, and blood samples were taken from each animal in a sterile gel tube. They were centrifuged for 2 hours at room temperature. An automated chemical analyzer removed the supernatant from the pellet and used it to

create serum samples for TG, total cholesterol, and HDL-C measurements, as needed.

Statistical Analysis

All of the tests were carried out three times independently and in triplicate. Expressed as mean minus standard deviation (SD). GraphPad Prism® 8.02 was used for statistical analysis, including one-way ANOVA and the Kruskal–Wallis test (GraphPad Software, Inc., San Diego, CA, USA), significantly different at *p<0.05, **p<0.01 and ***p<0.001 as compared to diabetic control group.

Results

Phytochemical investigation

Results showed that alkaloids, carbohydrates, tannins, glycosides, and flavonoids were present in the extract.

Antidiabetic activity of *C. indicum* flower extract

On STZ-induced diabetic mice, *C. indicum* floral extract showed anti-hyperglycemic action. The extract considerably (p<0.001) lowered the blood glucose levels of mice in a dose-dependent manner, according to the results. Compared to a diabetic control group, mice treated with *C. indicum* extract at doses of 200 mg/kg and 400 mg/kg had blood glucose levels on day 21 that were 92.11 ±5.71 mg/dL and 83.38 ±3.99 mg/dL, respectively. In diabetic rats, glibenclamide was most effective at lowering blood glucose levels to 81.31 ±3.01 mg/dL, the standard measurement. The baseline blood glucose of diabetic mice was considerably (p<0.001) higher than that of normal control mice in all groups of diabetic mice analysed across groups (Table 1). On the 7thand 14th days of treatment, the glibenclamide-treated group's blood glucose levels were significantly lower than those of the diabetes controls, as well. On the 14th day of treatment, 400 mg/kg *C. indicum* considerably (P<0.001) lowered blood glucose levels compared to the diabetic control group, according to the group analysis.

Antihyperlipidemic activity of *C. indicum* flower extract

Figure 1 reveals that *C. indicum* floral extract has antihyperlipidemic properties. The diabetic control had a substantial (p<0.001) increase in serum total cholesterol and triglycerides, while the normal control had a significant (p<0.001) decrease in HDL cholesterol (Table 2). When administered *C. indicum* 400 mg/kg daily for 21 days, the level of total cholesterol decreased (108.64 ± 5.94 mg/dL) and triglyceride increased (85.32 ±4.04 mg/dL) compared to the diabetic control, while the level of HDL cholesterol increased (38.76 ±3.04 mg/dL). There was a substantial reduction in blood cholesterol and triglyceride levels (p<0.001), and an increase in HDL cholesterol (p<0.01), with the conventional medication (glibenclamide).

Discussion

In the 21st century, diabetes is a major global health crisis (Standl et al., 2019). As currently existing DM drug regimens have limits, it is imperative that a better treatment option be developed that is also less harmful to patients. Compounds having anti-diabetic action and long-term safety should be sought

out by patients with coexisting diabetes and dyslipidemia. Thus, the study of plant-derived chemicals for the treatment of DM is an appealing research area since they are believed to be safe, accessible, and do not necessitate a lengthy pharmaceutical synthesis. The plant extract's LD50 is larger than 2000 mg/kg shows that it has a wide safety margin, according to this study.

It is well-known and confirmed that streptozotocin induces diabetes in rats. Toxic effects of STZ on pancreatic beta cells are thought to be mediated in part through DNA methylation, nitric oxide, and reactive oxygen species (ROS) (Eleazu et al., 2013). At a dosage of 150 mg/kg STZ, mice developed hyperglycemia that lasted for at least eight weeks after a single intraperitoneal injection.

There were no significant variations in baseline blood glucose across groups in this investigation as in all animal models. Similarly, in all animal models, there was no drop in blood glucose levels in the vehicle-treated groups compared to the baseline levels. However, after repeated daily dose administration of the plant extract, considerable reduction was found in the diabetic rats, demonstrating the change in blood glucose was due to the treatment received. Plant extracts may have anti-diabetic properties due to the presence of phytochemical components.

C. indicum's antihyperglycemic actions could be explained by the existence of these various secondary metabolites, which have been shown to have blood glucose-lowering properties and may even work in concert to reduce glucose levels even more effectively. Diabetes-induced glibenclamide-treated diabetic mice showed antihyperglycemic efficacy. Glibenclamide acts by selectively blocking ATP-sensitive K+ channels in the plasma membrane of pancreatic -cells, leading to cytosolic depolarization and endogenous insulin release (Alotaibi et al., 2019).

C. indicum's antihyperglycemic properties may be attributed to either an increase in insulin secretion from beta cells of the pancreas or an increase in the absorption of glucose by the peripheral tissues. There is still a need for extensive molecular investigations to determine the specific mechanism of *C. indicum's* antihyperglycemic effect. Animals that have been given STZ to induce experimental diabetes have lost a significant amount of weight. Mice with severe hyperglycemia in their blood following STZ treatment lose more weight, according to research.

One of the consequences of diabetes mellitus is lipid abnormalities, which is characterised by high blood TG, TC, and low HDL-C levels (Ozder, 2014). Increased lipolysis and liver VLDL secretion can occur as a result of insulin shortage activating hormone-sensitive lipase. Chylomicron and VLDL clearance is also reduced as a result of insulin insufficiency, which reduces lipoprotein lipase activity (Goldberg and Chait, 2020). Cholesteryl ester transfer protein is also stimulated by hypertriglyceridemia to increase the triglyceride content of LDL and HDL. Catabolism and hydrolysis of triglyceride-enriched HDL particles and triglyceride-enriched LDL particles result in lower LDL particle size, respectively.

Conclusion

Antihyperglycemic and antihyperlipidemic properties of C. *indicum* floral extract were found in this investigation. The study's findings provide scientific credence to folk medicine's usage of the herb to treat diabetes and the issues that come along with it. Clinical research could lead to its adoption as an alternate antidiabetic medication. To fully understand this plant's antidiabetogenic and antihyperlipidemic activities more research is required to get the bioactive compounds responsible for these properties.

Declaration

The authors declared no conflict of interest.

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Table 1 Antidiabetic activity of *C. indicum* flower extract

Treatment	Dose	0 Day (mg/dL)	7 Day (mg/dL)	14 Day (mg/dL)	21 Day (mg/dL)
Negative control	Saline 0.5 ml	78.38 ± 1.38	79.12 ± 2.20	79.30 ± 1.30	79.22 ± 2.49
Positive control	-	249.20 ± 9.20	242.23 ± 8.30	240.93 ± 8.92	240.10 ± 9.11
Reference	100 mg/kg	248.82 ± 7.88	148.11 ± 4.20	118.88 ± 5.93	81.31 ± 3.01 ***
C. indicum 1	200 mg/kg	250.39 ± 6.30	182.48 ± 5.30	148.19 ± 8.45	92.11 ± 5.71 **
C. indicum 2	400 mg/kg	248.19 ± 8.42	173.02 ± 6.23	129.66 ± 4.21	83.38 ± 3.99***

Data are represented as mean ± SD (n=6), significantly different at *p<0.05, **p<0.01 and ***p<0.001 as compared to diabetic control group.

Table 2 Antihyperlipidemic activity of *C. indicum* flower extract

Treatment	Dose	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)
Negative control	Saline 0.5 ml	85.11 ± 3.13	75.34 ± 2.97	42.86 ± 3.33
Positive control	-	199.75 ± 5.87	162.81 ± 6.93	20.43 ± 1.86
Reference	100 mg/kg	98.42 ± 2.92	78.56 ± 3.71	41.08 ± 2.41
C. indicum 1	200 mg/kg	130.22 ± 3.97	102.55 ± 3.75	33.88 ± 1.75 *
C. indicum 2	400 mg/kg	108.64 ± 5.94	85.32 ± 4.04	38.76 ± 3.04 **

Data are represented as mean ± SD (n=6), significantly different at *p<0.05, **p<0.01 and ***p<0.001 as compared to diabetic control group.

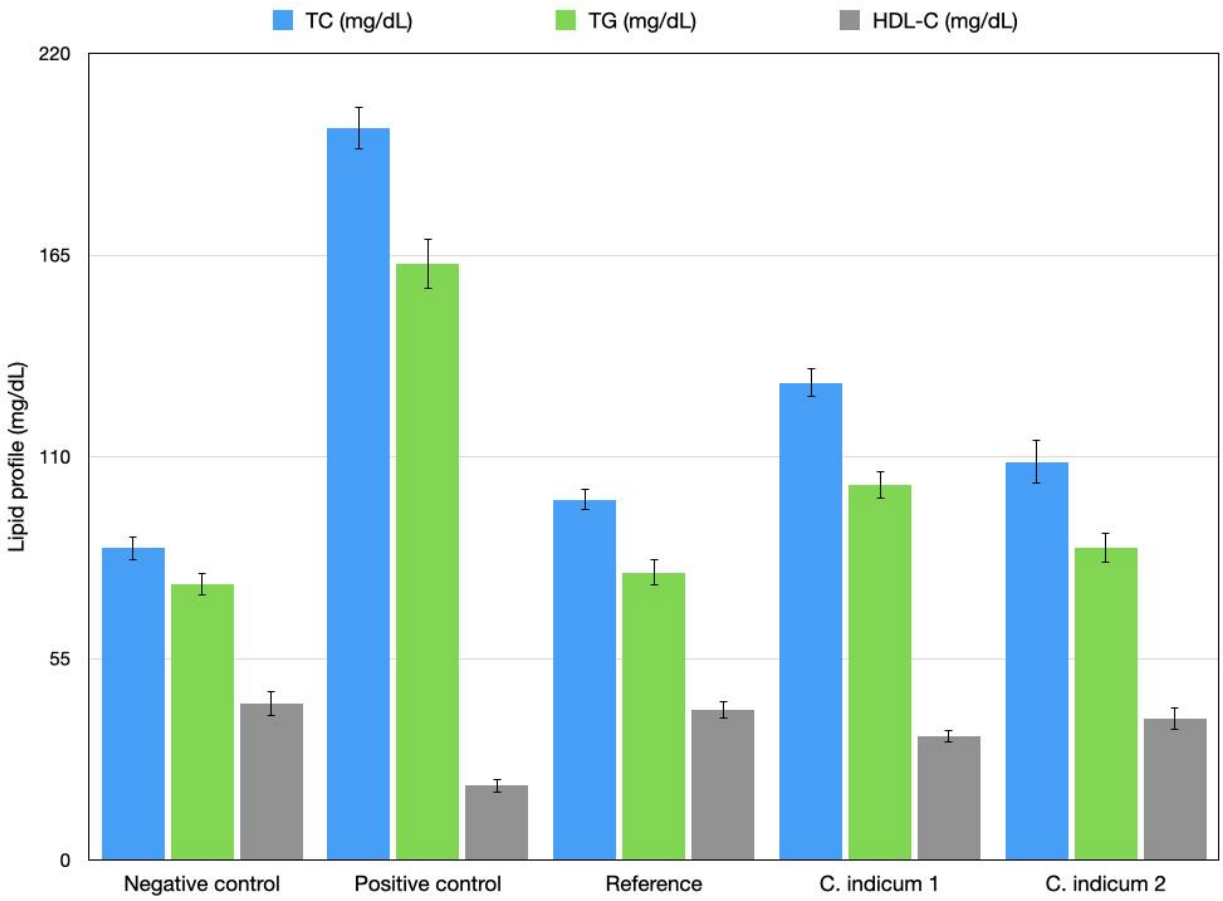

Figure 1 Effect of flower extract on lipid profile of STZ-induced diabetic rats