

Potential Antidiabetic Activity and Lipid Profile Effects of Plant Extracts in diabetic induced Wistar rats

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, which results from deficiencies in insulin secretion, insulin action, or both. To investigate the antidiabetic activity and lipid profiles, diabetic-induced Wistar rats were treated with plant extracts, and comparative studies were conducted. Diabetes was induced using a single intraperitoneal injection of Alloxan drug (120 mg/kg), and the results of the test drug were compared with the diabetic control group. Glibenclamide (600 µg/kg/bw) was used as the standard hypoglycemic drug. The administration of plant extracts demonstrated antidiabetic activity and reduction in blood glucose levels at the 7th day. Moreover, the administration of plant extracts led to a decrease in serum levels of TC, TG, HDL, and LDL in diabetic-induced Wistar rats. The selected plant extracts, including *Hemidesmus indicus*, *Cassia auriculata*, *Chloroxylon swietenia*, and *Tinospora cordifolia*, showed potential antidiabetic activity as revealed by the results of the study. The study findings suggest that these plant extracts could be used as an alternative or complementary treatment for diabetes mellitus. However, further studies are required to determine their safety and efficacy in humans. Future studies could focus on examining the pharmacological mechanisms of these plant extracts to provide a better understanding of their therapeutic potential.

Key Words: Traditional healers, Medicinal plants, Ethnobotanical survey, diabetes mellitus, lipid profile.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Diabetes is a major public health concern worldwide, affecting an estimated 463 million adults in 2019, with the number expected to rise to 700 million by 2045 (International Diabetes Federation, 2019). Uncontrolled diabetes can lead to serious complications like cardiovascular disease, kidney failure, blindness, and nerve damage.

Several natural products, including plants, have been investigated for their antidiabetic properties. Plant extracts are rich in bioactive compounds that can potentially regulate blood glucose levels and improve lipid profiles. In recent years, there has been an increasing interest in using plant extracts as a complementary and alternative therapy for diabetes. Plant extracts have been found to have various bioactive compounds such as polyphenols, flavonoids, and alkaloids that have antidiabetic and lipid-lowering effects. These bioactive compounds act through different mechanisms such as increased insulin sensitivity, inhibition of carbohydrate-digesting enzymes, and reducing oxidative stress (Mohamed et al., 2020).

The Wistar rat is a widely used experimental model for studying diabetes. The induction of diabetes in Wistar rats can mimic the pathophysiology of human diabetes, making it a useful tool for investigating potential antidiabetic agents. Studies have shown that various plant extracts have antidiabetic activity and may also have beneficial effects on lipid profiles. For example, a study by El-Halawany et al. (2021) investigated the antidiabetic and lipid-lowering effects of *Annona squamosa* (custard apple) leaf extract in diabetic rats and found significant reductions in blood glucose levels and improvements in lipid profiles. Similarly, a study by Roy et al. (2020) evaluated the antidiabetic and hypolipidemic effects of garlic extract in diabetic rats and found significant reductions in blood glucose levels and improvements in lipid profiles. A study by Chakraborty et al. (2021) investigated the antidiabetic effects of *Momordica charantia* (bitter melon) extract in diabetic rats and found significant reductions in blood glucose levels and improvements in lipid profiles. Another study by Ahmed et al. (2021) evaluated the antidiabetic and lipid-lowering effects of *Nigella sativa* (black seed) extract in diabetic rats and found significant reductions in blood glucose levels and improvements in lipid profiles.

Moreover, plant extracts are generally considered safe and have fewer side effects compared to synthetic drugs. Many plant extracts have been used traditionally for treating diabetes and have gained scientific attention due to their potential therapeutic benefits. This paper aims to investigate the potential antidiabetic activity and lipid profile effects of selected plant extracts in diabetic-induced Wistar rats. The findings of this study may contribute to the development of new and effective therapies for the management of diabetes and its associated complications.

MATERIALS AND METHODS

Chemicals:

Alloxan monohydrate and Glibenclamide were obtained from Sigma in Bangalore, while the Wellion LUNA duo Glucometer and Blood gluco-strips were procured from Med Trust-GlucoWorld in Kerala. Additionally, the following reagents were obtained from Sigma in Bangalore: EDTA, NaCl (0.9% w/v), Phosphate buffer with a pH of 6.5 at a concentration of 90 mmol/L, Phenol at a concentration of 26 mmol/L, 4-Aminoantipyrine at a concentration of 0.4 mmol/L, Cholesterol Esterase at a concentration of 500 U/L, Cholesterol Oxidase at a concentration of 500 U/L, Peroxidase at a concentration of 1250 U/L, and Glycerol kinase at a concentration of 1250 U/L. All of the aforementioned reagents are ready for use and exhibit stability up to the expiry date specified on their respective labels when stored at a temperature range of 2-8°C.

Preparation of Extracts:

The plant powders were extracted using the Soxhlet extraction method in which successive extractions were carried out using different organic solvents in increasing polarity order, as per Manjula et al., (2013). The extraction process involved placing 500 grams of the powdered material in the Soxhlet extraction chamber, which was suspended above a flask containing 1000 ml of 80% solvent and below a condenser. The solvent was heated, evaporated and moved into the condenser, where it turned into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed to prevent overflowing of the solvent surrounding the sample. The solvents used in increasing polarity order were n-Hexane, Chloroform, Ethyl acetate, Acetone, and Methanol. After extraction, the excess solvent was removed using a rotary evaporator, and the weight of the residual extract was measured to calculate the percent yield. The residue of the extract was then dissolved in 25 ml of pure methanol and stored in air-tight glass vials at a temperature of 4°C until further use.

Extract yield % = $W1/W2 \times 100$;

Where, W1 = Net Weight of powder in grams after extraction,

W2 = total Weight of powder in grams taken for extraction

Test Animals:

For the study, adult albino Wistar rats aged between 8-14 weeks and weighing 150-155 grams were allowed to acclimatize for a period of 7 days before the beginning of the experiment. They were housed in groups of seven in polypropylene cages with soft wood shavings as bedding, which were renewed every 24 hours. The rats were maintained under 12/12 hours light/dark cycles, with a relative humidity of 50-60% and a temperature of $22\pm3^{\circ}\text{C}$. They were provided with rat pellet diet (Gold Moher, Lipton India Ltd) and water ad libitum on a regular basis.

Experimental design:

The animals for the experiment were selected and fasted overnight. They were then divided into seven groups (n=6) as described below:

Group-I: Normal Control rats (non-alloxanized) that were given standard feed and water.

Group-II: Diabetic Control rats (Untreated, alloxanized).

Group-III: Diabetic rats were administered Glybenclamide drug ($600\mu\text{g/kg/bw}$) as a reference standard.

Group-IV: Diabetic rats were administered *Hemedesmus indicus* leaf extract (11 mg/bw).

Group-V: Diabetic rats were administered *Cassia auriculata* leaf extract (7 mg/bw).

Group-VI: Diabetic rats were administered *Chloroxylon swietenia* bark extract (8 mg/bw).

Group-VII: Diabetic rats were administered *Tinospora cordifolia* leaf extract (10 mg/bw).

Induction of experimental diabetes:

To induce diabetes, normal rats were fasted overnight and then given a single intraperitoneal injection of alloxan monohydrate (120 mg/kg b.w.) dissolved in 0.9% w/v NaCl solution (normal saline). After 72 hours of alloxanisation, the blood glucose levels were checked using a one-touch glucometer to confirm diabetes. Rats with fasting blood glucose (FBG) levels greater than 250 mg/dL were selected for the study. The treatment was administered orally to the experimental animals using a force-feeding needle and was continued for a period of 7 days.

Specimen Collection and Preparation:

The specimens used for the study were serum samples and EDTA that were free from hemolysis. The serum was separated from the blood cells. The stability of total cholesterol in serum was found to be at least 7 days when stored at a temperature range of $2-8^{\circ}\text{C}$, up to 3 months when stored at $\leq -20^{\circ}\text{C}$, and at -70°C , as per Luthra et al., (2017). The estimation of cholesterol, triglyceride, HDL, and LDL in serum was performed on the 0th day and the 7th day after completion of the treatment for all groups of animals.

Estimation of Serum Lipids by CHOD-PAP-Phosphotungstate method:

The activity of cholesterol, triglyceride, HDL, and LDL in serum was quantitatively determined in vitro using the CHOD-PAP method, as described by Poojari et al., (2014) and Gujjeti et al., (2014).

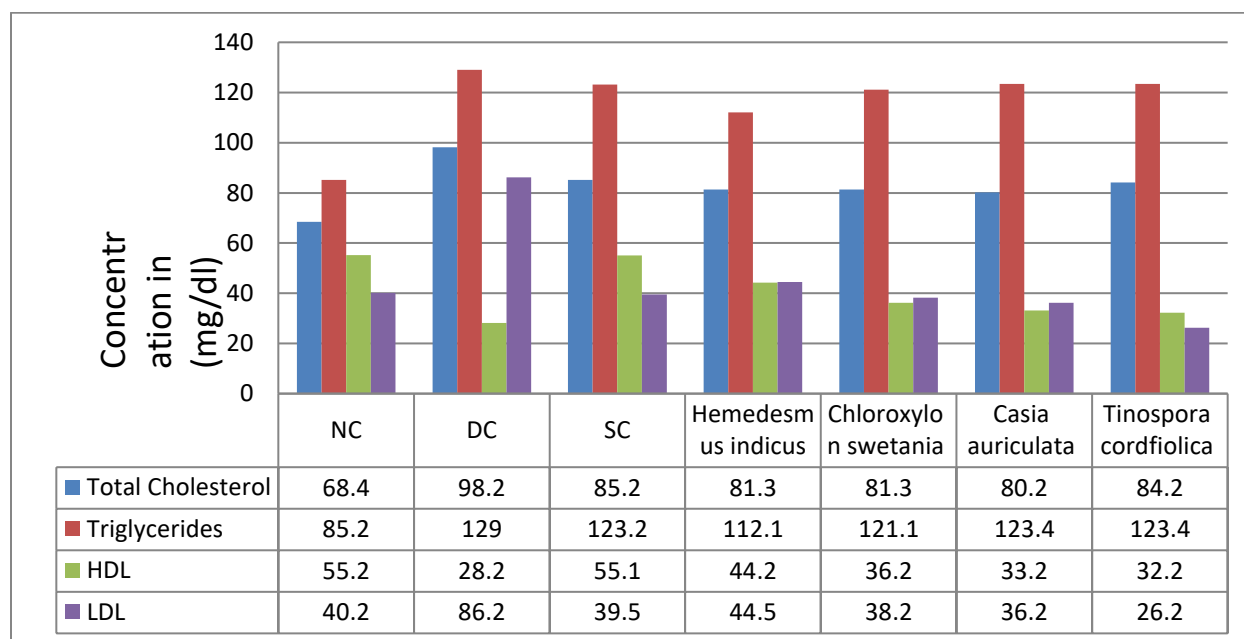
RESULTS AND DISCUSSION

Total cholesterol:

The normal control group rats had a level of 68.4mg/dL, the diabetic control group rats had a level of 98.2mg/dL, the standard control group rats had a level of 85.2mg/dL. The *Hemedesmus indicus* leaf extract treated group rats had a level of 81.3mg/dL, *Chloroxylon swietenia* bark extract treated group rats had a level of 81.3mg/dL, *Cassia auriculata* flower extract treated group rats had a level of 80.2mg/dL,

and *Tinospora cordifolia* leaf extract treated group rats had a level of 84.2mg/dL. Comparison of the lipid profile in diabetic-induced rats treated with plant extracts and controls was depicted Figure-1.

Figure-1. Lipid Profile comparison in diabetic induced rats treated with plant extract and controls



Triglycerides:

The normal control group rats had a level of 85.2mg/dL, the diabetic control group rats had a level of 129mg/dL, the standard control group rats had a level of 123.2mg/dL. The *Hemedesmus indicus* leaf extract treated group rats had a level of 112.1mg/dL, *Chloroxylon swietenia* bark extract treated group rats had a level of 121.1mg/dL, *Cassia auriculata* flower extract treated group rats had a level of 123.4mg/dL, and *Tinospora cordifolia* leaf extract treated group rats had a level of 113.4mg/dL.

HDL:

The normal control group rats had a level of 55.2mg/dL, the diabetic control group rats had a level of 28.2mg/dL, the standard control group rats had a level of 55.1mg/dL. The *Hemedesmus indicus* leaf extract treated group rats had a level of 42.2mg/dL, *Chloroxylon swietenia* bark extract treated group rats had a level of 36.2mg/dL, *Cassia auriculata* flower extract treated group rats had a level of 33.2mg/dL, and *Tinospora cordifolia* leaf extract treated group rats had a level of 32.2mg/dL.

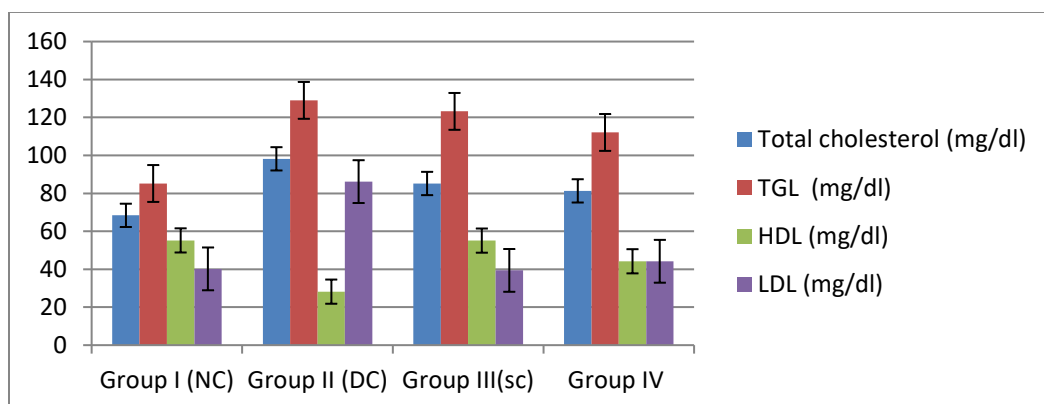
LDL:

The normal control group rats had a level of 40.2mg/dL, the diabetic control group rats had a level of 86.2mg/dL, the standard control group rats had a level of 39.4mg/dL. The *Hemedesmus indicus* leaf extract treated group rats had a level of 44.2mg/dL, *Chloroxylon swietenia* bark extract treated group rats had a level of 38.2mg/dL, *Cassia auriculata* flower extract treated group rats had a level of 36.2mg/dL, and *Tinospora cordifolia* leaf extract treated group rats had a level of 26.2mg/dL.

Table-1: Effect of *Hemedesmus indicus* leaf extract on Lipid profile of Wistar rats

	Total cholesterol (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group I (NC)	68.4±4.02	85.2±6.02	55.2±0.48	40.2±1.01
Group II (DC)	98.2±1.6	129±1.58	28.2±0.78	86.2±2.0
Group III(sc)	85.2±1.12	123.2±1.52	55.1±1.67	39.4±3.12
Group IV <i>Hemedesmus indicus</i> (11mg/bw)	81.3±1.18	112.1±2.01	44.2±1.31	44.2±2.12

Figure-2: Effect of *Hemedesmus indicus* leaf extract on Lipid profile of Wistar rats



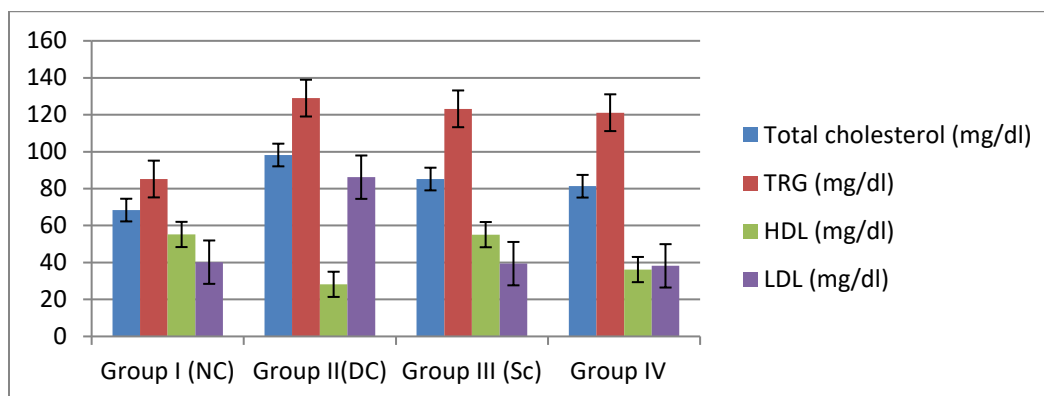
The table-1 and Figure-2 above illustrate the impact of *Hemedesmus indicus* leaf extract on the lipid profile of Wistar rats. The results show that the treatment resulted in a 17.3% reduction in total cholesterol compared to the diabetic control group rats. The triglycerides were also reduced by 13.11% compared to the diabetic control group rats. Additionally, HDL levels increased by 56.73% compared to the diabetic control group rats, while LDL levels decreased by 51.27% compared to the diabetic control group rats.

Table 2: Effect of *chloroxylon swetania* bark extract on lipid profile of wistar rats

	Total cholesterol (mg/dl)	TRG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group I (NC)	68.4±4.02	85.2±6.02	55.2±0.48	40.2±1.01
Group II(DC)	98.2±1.6	129±1.58	28.2±0.78	86.2±2.0
Group III (Sc)	85.2±1.12	123.2±1.52	55.1±1.67	39.4±3.12

Group IV <i>Chloroxylon swetania</i> (8mg/bw)	81.3±1.14	121.1±1.48	36.2±0.41	38.2±0.15
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Figure-3: Effect of *chloroxylon swetaniabark* extract on lipid profile of wistar rats

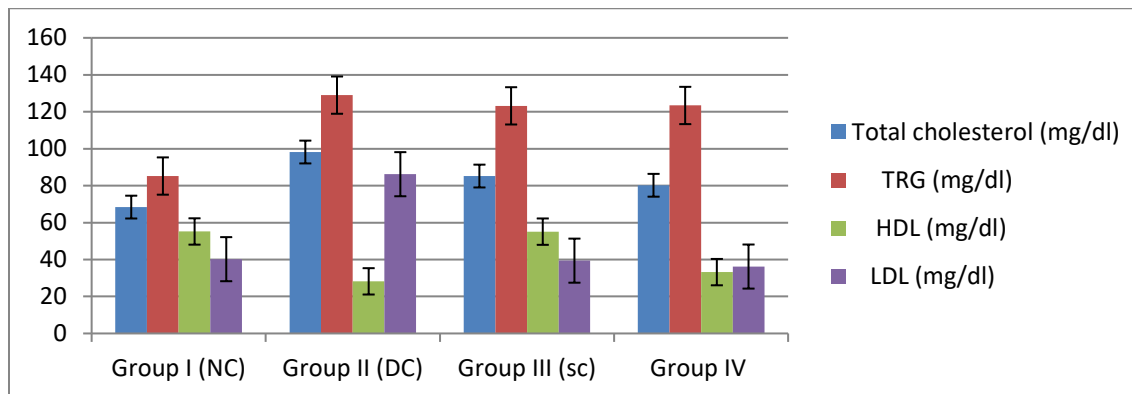


The table-2 and Figure-3 above display the impact of *Chloroxylon swietenia* bark extract on the lipid profile of Wistar rats. The results indicate that the treatment led to a 17.21% decrease in total cholesterol compared to the diabetic control group rats, as well as a 6.13% decrease in triglycerides compared to the diabetic control group rats. Moreover, the treatment resulted in a 28.36% increase in HDL compared to the diabetic control group rats, and a 44.31% decrease in LDL compared to the diabetic control group rats.

Table 3: Effect of *casia auriculata* flowerextract on lipid profile of wistar rats

	Total cholesterol (mg/dl)	TRG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group I (NC)	68.4±4.02	85.2±6.02	55.2±0.48	40.2±1.01
Group II (DC)	98.2±1.6	129±1.58	28.2±0.78	86.2±2.0
Group III (sc)	85.2±1.12	123.2±1.52	55.1±1.67	39.4±3.12
Group VI <i>Casia auriculata</i> (7mg/bw)	80.2±1.2	123.4±1.51	33.2±1.42	36.2±2.98

Figure-4: Effect of *Cassia auriculata* flower extract on lipid profile of wistar rats

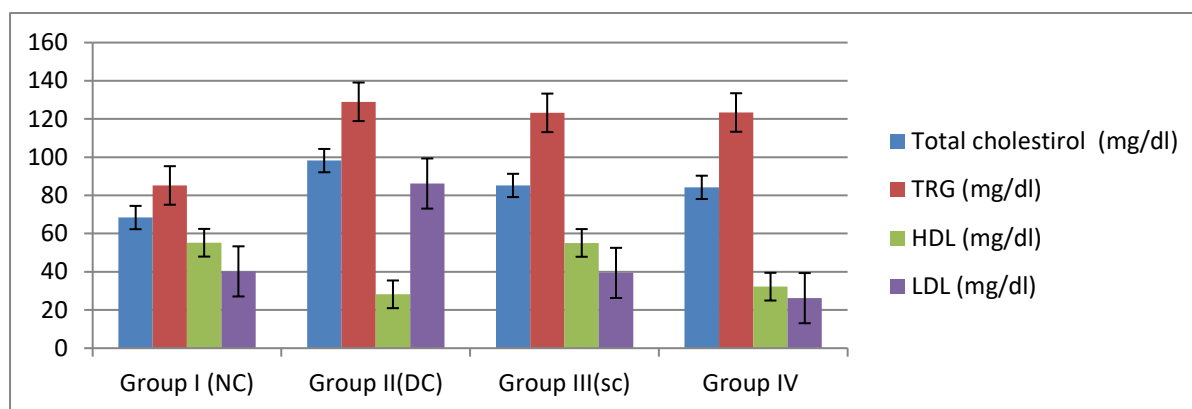


The table-3 and Figure-4 above demonstrate the impact of *Cassia auriculata* flower extract on the lipid profile of Wistar rats. The outcomes reveal that the treatment resulted in an 18.33% decrease in total cholesterol compared to the diabetic control group rats, along with a 4.35% decrease in triglycerides compared to the diabetic control group rats. Furthermore, the treatment led to a 17.73% increase in HDL compared to the diabetic control group rats, and a 41.99% decrease in LDL compared to the diabetic control group rats.

Table 4: Effect of *Tinospora cordifolia* leaf extract on lipid profile of wistar rats

	Total cholestirol (mg/dl)	TRG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group I (NC)	68.4±4.02	85.2±6.02	55.2±0.48	40.2±1.01
Group II(DC)	98.2±1.6	129±1.58	28.2±0.78	86.2±2.0
Group III(sc)	85.2±1.12	123.2±1.52	55.1±1.67	39.4±3.12
Group VII <i>Tinospora cordifolia</i> (10mg/bw)	84.2±1.13	123.4±1.51	32.2±1.01	26.2±2.02

Figure-5: Effect of *Tinospora cordifolia* leaf extract on lipid profile of wistar rats



The results of the research revealed that *Tinospora cordifolia* leaf extract had a significant impact on the lipid profile of Wistar rats, as shown by the table-4 and Figure-5. The treatment resulted in a 14.26% reduction in total cholesterol and a 4.35% decrease in triglycerides compared to the diabetic control group rats. Additionally, the treatment led to a 14.18% increase in HDL and a 30.16% decrease in LDL compared to the diabetic control group rats. The study confirmed the induction of diabetes in rats by observing elevated levels of fasting blood glucose. The primary aim of the study was to investigate the effects of selected plant extracts, including *Hemidesmus indicus*, *Cassia auriculata*, *Chloroxylon swietenia*, and *Tinospora cordifolia*, on the lipid profile of diabetic rats.

The present study's results demonstrate that multiple doses of plant extracts effectively decrease fasting blood glucose levels in Alloxan-induced diabetic rats. The mechanism of Alloxan-induced diabetes is still not fully understood, but studies suggest that it involves the accumulation of cytotoxic free radicals leading to the degeneration of islet-cells (Halliwell et al., 1989; Vijayagiri et al., 2012; Mamidala et al., 2013). In this study, the plant extracts were administered daily for 7 days, resulting in a significant reduction in all diabetic markers. This effect was more potent than acute dosing. Elevated blood glucose levels are often accompanied by increased Total Cholesterol (TC), Triglycerides (TG), Low-Density Lipoprotein (LDL), and decreased High-Density Lipoprotein (HDL) levels. Abnormal lipid metabolism is a known predictor of diabetes mellitus (Shukla et al., 1995). The administration of plant extracts caused a significant reduction in serum levels of TC, LDL, and TG, while also elevating HDL levels. The 7-day treatment resulted in the restoration of all these parameters to normal levels. Additionally, the administration of plant extracts to diabetic rats significantly decreased blood glucose levels and increased serum insulin levels. Furthermore, the activity of catalase in diabetic rats was augmented, which could be attributed to the strong antidiabetic property of plant extracts.

CONCLUSIONS

The present study investigated the effects of administering selected plant extracts, namely *Hemidesmus indicus*, *Cassia auriculata*, *Chloroxylon swietenia*, and *Tinospora cordifolia*, on diabetic-induced Wistar rats. The findings revealed a permanent reduction in blood glucose levels and normalization of lipid profiles compared to the diabetic control group. Based on the results, it can be concluded that the selected plant extracts are remarkably effective against Alloxan-induced diabetes in Wistar rats.

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