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RESEARCH PAPER

# Phytochemical analysis and hypolipidaemic activity of *Cestrum nocturnum* (Solanaceae: Solanales) extract in normal albino rat

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**Abstract**
During the present study, we investigated the hypolipidemic activity of chloroform: methanol extract of green leaves of *Cestrum nocturnum* (Solanaceae: Solanales) in normal albino rats. Phytochemical analysis of the plant extract revealed the presence of essential oil, bi-terpenoids, steroids, protein, phenolics and alkaloids. During the Hematological experiment, normal albino rats were administered a single dose of plant extract (20 mg/ kg body weight/day) for up to fifteen days. In plant extract administered to albino rats, a significant reduction in serum total cholesterol level was observed. The plasma triglyceride levels also significantly decreased. A sharp increase in the HDL Cholesterol level and a substantial decrease in the LDL and VLDL amount were also observed. The result of the atorvastatin-treated rats also showed similar results to that of plant extract-administered rats. A sharp increase in the HDL Cholesterol level and a significant decrease in the LDL and VLDL amount in plant extract administered at a normal albino rate in comparison to control rats established the hypolipidemic activity of the tested dose of plant extract in the experimental animal body.

**Keywords** High-density lipoprotein cholesterol · Low-density lipoprotein cholesterol · Plant extract · Triglycerides · Very low-density lipoprotein cholesterol

**Abbreviations**

| | |
|---|---|
| CAD | Coronary artery diseases |
| HDL | High-density lipoprotein cholesterol |
| LDL | Low-density lipoprotein cholesterol |
| VLDL | Very low-density lipoprotein cholesterol |

## Introduction

The incidence of cardiovascular diseases is rising continuously around the world. Most cardiovascular diseases are linked to conditions such as coronary artery diseases (CAD), stroke, and hypertension. Hyperlipidemia, a condition characterized by elevated levels of plasma lipids, is a major risk factor for cardiovascular diseases. Excess lipids accumulate in the arterial walls, restricting blood flow to vital organs and leading to conditions such as hypertension. The primary plasma lipids involved in these complications are cholesterol and triacylglycerols.

Among the different types of cholesterol, high-density lipoprotein (HDL) is considered "good" cholesterol because it helps transport lipids from blood cells to the liver. In contrast, low-density lipoprotein (LDL) is known as "bad" cholesterol, as it moves lipids toward cells and blood vessels, contributing to plaque buildup. The rise in hyperlipidemia-related diseases is closely linked to shifts in modern dietary habits. This metabolic disorder can be traced to disruptions in lipid biosynthesis, absorption, transport, and excretion. Globally, hyperlipidemia is responsible for over 17 million deaths each year (WHO 2011). The condition is primarily associated with decreased serum HDL levels and elevated levels of total cholesterol (TC), triglycerides (TG), and LDL cholesterol (LDL-C) (Gambhir et al. 2001). It is a major risk factor for diseases like nonalcoholic fatty liver disease, atherosclerosis, diabetes, stroke, heart attacks, and acute pancreatitis.

For years, researchers have been focused on finding effective ways to lower cholesterol levels. Existing treatments fall into three main categories: natural remedies, synthetic lipid-lowering drugs, and lifestyle modifications. The most

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prescribed hypolipidemic drugs include fibrates, niacin, statins, and cholesterol absorption inhibitors. While effective, these medications can cause serious side effects, such as diabetes, muscle breakdown (rhabdomyolysis), liver damage, and even cancer. Some also come with withdrawal symptoms, limiting their long-term use (Ma 2018).

Because of these concerns, there is growing interest in plant-based alternatives (Ansari et al. 2020). Many traditional medicinal plants contain bioactive compounds—such as phytosterols, polyphenols, alkaloids, terpenoids, and polysaccharides—that have been shown to lower cholesterol with fewer side effects (Cheng et al. 2020; Feng et al. 2018). Clinical studies suggest that these natural compounds can be just as effective, or even more so, than conventional drugs (Nechchadi et al. 2024).

*Cestrum nocturnum* L. (Solanaceae), commonly known as night-blooming Jessamine, has demonstrated antibacterial properties in its aqueous and methanolic extracts against various bacteria including *Staphylococcus aureus* (MTCC 2940), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 739), and *Pseudomonas aeruginosa* (MTCC 2453) (Chatterjee et al. 2007). Flavone glycosides and steroidal glycosides have been isolated from this plant, and their effects on cytotoxic activities against human oral squamous cell carcinoma (HSC-2) and normal human gingival fibroblasts have been documented (Mimaki et al. 2001).

The current study aims to investigate the hypolipidemic activity of the chloroform: methanol extract of *Cestrum nocturnum*. Additionally, this study will include a phytochemical analysis of the *Cestrum nocturnum* to provide initial insights into the bioactive compounds present in the plant extract.

## Materials and methods

### Plant material

During the present study, for preparation of plant extract**,** two hundred grams of fresh, mature, green leaves of *C. nocturnum* were randomly harvested during August and September 2018, from plants growing on the outskirts of Burdwan University, West Bengal, India. The respective plant was identified in the Department of Botany, Burdwan University, and a voucher specimen (GC-CESN/2018/21) was kept in the Department of Zoology, Burdwan University as a voucher specimen.

## Preparation of plant extract

All the collected leaves were initially rinsed with distilled water and dried on a paper towel. The dried leaves were put in a Soxhlet apparatus and the plant extracts were prepared using 750 ml of chloroform: methanol (1:1 v/v) solvent (extraction period 72 h and the temperature was < 40 °C). The extract was concentrated at 40 °C to 200 ml of extract by evaporation in a rotary evaporator. Then the extract was filtered, the solvents were evaporated by lyophilizer and the solid residues were weighed.

### Phytochemical analysis of the plant extract

Physico-chemical constants such as moisture content, ash content, total protein, soluble carbohydrates, starch content, phenolic content and lipid contents of the collected fresh mature leaves were quantitatively calculated according to suitable methodologies (Ghosh and Chandra 2005). The phytochemical analysis was carried out using the chloroform: methanol extract using the Thin Layer Chromatography analysis (TLC) and the application of standard spraying reagents as described by Harbone 1984. The phytochemicals included under study were—saponins [Frothing test], sapogenins [Antimony chloride spray; Acetone: Hexane 1:4 as solvent], steroid and terpenoids [Leibermann Burchard], flavonoids [Saturated alcoholic sodium acetate spray; Chloroform–Acetic acid–Water (90:45:6) as solvent], alkaloid [Dragendroffs test], essential oils [Vanillin- Sulphuric acid spray; Chloroform-Benzene (1:1) solvent] phenolics (Ferric chloride test) and amino acids (Nin hydrin test).

### Study of hypolipidemic activity in albino rats

For the hematological experiment, the plant extract was again prepared through the Soxhlet extraction procedure described earlier. The extract was lyophilized in a rotary lyophilizer in the laboratory to get a solid residue of the extract that is used to establish the hypolipidemic activity of the extract in normal albino rats. For hematological examination, experiments were performed with twenty (20) albino rats of either sex weighing from 200 to 220 g. They were grouped into 4 sets as shown in Table 1. The rats were housed under standard environmental conditions ($23 \pm 10$ °C, with $65 \pm 5$% humidity), and maintained with free access to water and a standard laboratory diet. 5 rats from each group were housed in a cage having sufficient space. Before the treatment, the hematological parameters were evaluated from five experimental rats after three days of acclimatization period (Group 1). During the experimentation, each of the five rats of the control set (Group 2) was allowed to take normal feed and the rats of the Atorvastatin treated sets (Group 3) received Atorvastatin (5 mg/kg body weight/day) along with food and water and the last set (Group 4) having five rats got an oral administration of single dose of plant extract (20 mg/ kg body weight/day) up to fifteen days along with their normal diet after which all the rats were sacrificed to analyze the hematological parameters

**Table 1** Change in the lipid profile (mean ± standard error) of albino rats following oral administration of steroid compound

| Hematological parameters | Before experimentation (Group I) | After experimentation | | |
|---|---|---|---|---|
| | | Control rats (Group 2) | Atorvastatin treated rats (Group 3) | Plant extract treated rats (Group 4) |
| WBC ($10^3$/μL) | 6.533 ± 0.088 | 6.50 ± 0.12 | 6.36 ± 0.088 | 6.43 ± 0.033 |
| Serum triglycerides | 76.33 ± 1.453 | 77.66 ± 1.764 | 57.33 ± 2.028 | 64.67 ± 1.202 |
| Serum total cholesterol | 81.33 ± 2.963 | 83.67 ± 2.189 | 67.00 ± 1.527 | 76.67 ± 1.856 |
| HDL cholesterol | 27.67 ± 1.764 | 28.33 ± 1.202 | 34.33 ± 1.453 | 32.67 ± 2.186 |
| LDL cholesterol | 28.33 ± 0.882 | 29.28 ± 1.155 | 24.86 ± 0.882 | 27.84 ± 0.333 |
| VLDL cholesterol | 18.33 ± 0.667 | 19.67 ± 0.333 | 15.33 ± 0.667 | 17.333 ± 0.333 |

(by cardiac puncture). Determination of total and free cholesterol in the serum was done by the method of Searcy and Berquist 1960. Serum triglyceride (TG) was determined using the method of Tiez 1990 after enzymatic hydrolysis by lipases LDL and HDL-cholesterol as well as VLDL- cholesterol was determined according to the methods described by Friedwald et al. 1972. The total white blood cell (WBC) counts were estimated using a Hemocytometer. All the studies were conducted according to the National Institute of Health guide, 1985 and the Burdwan University guide for the care and use of laboratory animals was followed.

## Result and discussion

The Results of the present study showed that the number of carbohydrates: 9.81 ± 0.06 mg/g, protein: 7.7 ± 0.06 mg/g, lipids: 258.95 ± 0.96 mg/g, phenolics: 0.36 ± 0.01 mg/g and starch: 18.03 ± 0.22 mg/g in matured leaves of *C. nocturnum*, The results of the preliminary phytochemical analysis indicated the presence of essential oil (Rf value 0.98), bi-terpenoid (0.94) flavonoids (0.89), steroid (0.96) protein (0.78), phenolics (0.85) and alkaloids (0.78) following the application of different solvent systems and spraying reagents.

Numerous studies have documented the hypolipidemic action of phytochemicals. By disrupting the process of cholesterol manufacture, for instance, steroidal saponins have been shown to lower blood cholesterol (Vijaya et al. 2009; Shi et al. 2014). Additionally, it was noted that tannins and polyphenols had hypoglycemic, hypolipidemic, and anti-obesity properties (Yin et al. 2011). Flavonoids are another secondary phytochemical that has been demonstrated to boost the body's resilience to LDL oxidation and decrease LDL levels (Kaamanen et al. 2003).

The change in the hematological parameters of control, treated and plant extract-treated albino rats are presented in Table 1. In plant extract administered to albino rats, a significant reduction in serum total cholesterol level was observed. The plasma triglyceride levels also significantly decreased. The WBC Count in the normal and treated rats was almost similar. A sharp increase in the HDL Cholesterol level and a significant decrease in the LDL and VLDL amount were also documented. However, no such significant change was observed between the control set and the rats sacrificed before experimentation. The result of the atorvastatin-treated rats also showed similar results to that of plant extract-administered rats.

The present study revealed that serum low-density lipoprotein (LDL) in the plant extract administered group was lower as compared with the control group. The HDL level also significantly increased in the plant extract administered group as compared with the control group that are suggestive of the hypolipidaemic effect of the plant extract at the tested doses. Previous studies also show that it is essential to have higher plasma HDL and lower LDL to prevent atherogenesis as described by Oyewole and Akingbala. 2011; Mushtaq et al. 2016; Hetta et al. 2017; Vengerovskii et al. 2019, Samal et al. 2023 etc. Furthermore, a previous study on HDL intervention reported 1% increase in HDL was associated with a 3% reduction in the risk of coronary heart disease and developing clinical atherosclerosis, according to Bainton et al. 1992.A reduction of plasma triglyceride levels also decreases the rate of coronary artery disease as described by Pradhan et al. 2020, and Gotto and Phil 1998.

The underlying mechanism of the hypo-lipidemic activity could be the inhibition of lipid absorption due to the presence of steroidal saponins in the chloroform: methanol extract. Ghosh et al. 2017 showed that the presence of phytosterol reduces the absorption of cholesterol in animals, the unutilized sterol moves into the intestinal lumen by 'sterolin pump'. In the intestinal lumen, the sterol component becomes converted to coprosterol and eliminated through faces (Juste and Gerrard 2021, Kriaa et al. 2019). So, there is less concentration of sterol compound available in the blood which reduces the risk of cardiovascular diseases.

## Conclusion

From the analysis of the result of the present experiment, it can be concluded that the chloroform: methanol extract of *C. nocturnum* extract caused a hypolipidaemic effect at the tested doses on normal albino rats. Further studies are suggested to confirm the bioactive compound responsible for the lipid-lowering activity of the plant extract and the mechanism of action of the bioactive principle in the animal body.

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**Author contributions** IB conducted the hematological experiments in laboratory conditions. SKC carried out the phytochemical analysis of the plant extract. AG planned and analyzed experimental data. GC finalized the experimental protocol and prepared the manuscript. The authors read and approved the final manuscript.

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**Data availability** All data generated and analyzed for this study are presented in the manuscript, and the corresponding author has no objection to the availability of data and materials.

## Declarations

**Competing interests** It is declared that the authors have no competing interests.

**Ethical approval** The study was conducted using the guidelines of the Dissection Monitoring Committee, Department of Zoology, The University of Burdwan [Established vide UGC, Ref No. F. 14-6/2014(CPP-II)].

**Consent for publication** The authors agree to publish this paper. The data has not been published partially or completely in any other journal.

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