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Research

Evaluation Of Anti Hyperlipidemic Activity Of *Sphaeranthus Indicus* Leaf Extract On Dexamethasone-Induced Hyper Lipidemia In Rats.

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Abstract

Siddha is one of the oldest medical systems in India, regarded as the foundational medicine of the ancient Tamils and Dravidians in South India. This system not only holds the distinction of being the most ancient but also boasts numerous specialties that surpass those found in Ayurvedic medicine. *Sphaeranthus indicus* Linn, belonging to the Asteraceae family, is commonly referred to by various names including Munditika, Mundi, Shravana, Bhikshu, Tapodhana, Mahashravani, Shravanahva, and Shravanashirshaka. This plant thrives in moist environments, particularly in plains, and is often found as a weed in rice paddies. In traditional Indian medicine, various parts of the plant such as the leaves, stems, bark, roots, flowers, and seeds are utilized for the treatment of numerous ailments. The plant is characterized by its bitter taste and is known for its stomachic, restorative, alterative, pectoral, demulcent, and soothing properties when applied externally. Research has identified a range of secondary metabolites present in the entire plant and its parts, including eudesmanolides, sesquiterpenoids, sesquiterpene lactones etc. In summary, our findings indicate that Sphaeranthus indicus effectively reduces oxidative stress through free radical scavenging offers protection against lipid peroxidation, and helps regulate hyperlipidemia by lowering serum cholesterol and triglyceride levels, comparable to standard medications. Additionally, phytochemical analyses have indicated the presence of Alkaloids, flavonoids, and Saponins.

Keywords: Sphaeranthus indicus, Antihyperlipidemic activity, Dexamethasone-induced hyperlipidemia, Flavonoids, Free radical scavenging, Wistar rats

INTRODUCTION

In wealthy societies, hyperlipidemia, commonly known as dyslipidemia, is common and a major risk factor for coronary heart disease. It is caused by abnormalities in the production, metabolism, or transportation of lipids and plasma lipoproteins. Elevated blood fat levels, especially triglycerides and cholesterol, are a hallmark of hyperlipidemia. Even while these fats are necessary for many bodily processes, too much of them might cause heart problems.

Hypercholesterolemia is the most prevalent kind of hyperlipidemia. While cholesterol is essential for the formation of cell membranes and the manufacturing of steroid hormones, triglycerides are used as energy sources and are carried in the circulation as lipoproteins. Atherosclerosis, heart attacks, and strokes are linked to dyslipidemia, which is characterized by elevated levels of total cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and triglycerides together with decreased HDL.

About two-thirds of the body's total cholesterol is produced in the liver and is controlled by the enzyme HMG-CoA reductase. Dietary and activity changes, lipid-lowering drugs such statins (HMG-CoA reductase inhibitors), bile acid sequestrants, fibrates, niacin, omega-3 fatty acids, and cholesterol absorption inhibitors are some of the treatment options for hyperlipidemia. But not all lipid problems can be treated by these medications, and many of them have negative side effects.

Because hyperlipidemia is asymptomatic, it frequently goes undiagnosed and is typically found during routine examinations. Genetics, a diet high in saturated fat, obesity, insulin resistance, or underlying diseases like diabetes can all contribute to it. Early onset is possible for familial variants such mixed hyperlipidemia and hypercholesterolemia, which frequently don't improve with lifestyle modifications alone. Atypical lipid profiles in children and adolescents might also be brought on by drugs, inadequate diet, or underlying medical conditions.

Pathophysiology

The main cause of hypercholesterolemia is poor lipoprotein metabolism, more especially, decreased LDL receptor expression or function, which restricts the liver's ability to remove LDL from plasma. This malfunction contributes to the development of atherosclerosis by causing lipid buildup. Serum triglyceride levels are also raised by increased VLDL synthesis, which is frequently observed in familial combination hyperlipidemia or insulin resistance situations (such as Type 2 diabetes and abdominal obesity). Because it stimulates inflammation, endothelial dysfunction, and the production of foam cells, oxidized LDL is essential to atherogenesis. The risk of thrombosis and abrupt cardiac events is raised when foam cells build up and burst within arterial plaques.

Need for natural hypolipidemic substances

Even while statins work, prolonged usage of them might have negative side effects such myopathy, rhabdomyolysis, and even renal failure. Interest in natural alternatives has increased due to worries about their side effects, cost, and safety. Hypolipidemic drugs derived from plants present a viable, less harmful alternative. Bioactive substances like flavonoids and saponins, which are frequently included in these natural products, aid in controlling lipid metabolism by preventing the absorption and production of cholesterol and by promoting HDL-mediated cholesterol transport. Thus, research into medicinal plants' capacity to reduce cholesterol may result in the creation of safer and more long-lasting treatments for hyperlipidemia.

Activity of antioxidants

Lipids, proteins, DNA, and cell membranes can all sustain oxidative damage from free radicals, which are atoms or molecules containing an unpaired electron. Numerous diseases, including diabetes, cancer, arthritis, atherosclerosis, and neurological disorders, are linked to the pathophysiology of reactive oxygen species (ROS), which include superoxide anions, hydrogen peroxide, and hydroxyl radicals. By chelating metal ions or contributing electrons, antioxidants counteract free radicals. Natural antioxidants found in medicinal plants are abundant, particularly phenolic substances with potent radical scavenging properties such as flavonoids, tannins, and polyphenols. These substances have anti-inflammatory, anti-carcinogenic, and cardio-protective qualities in addition to lowering oxidative stress. Natural antioxidants are becoming more and more popular in the food and pharmaceutical industries as a result of the shortcomings of synthetic preservatives and the rising demand for clean-label products. Their therapeutic potential in preventing chronic diseases is highlighted by their capacity to balance lipids, stop cellular damage, and boost immunological function.

Scope and work plan

Plan and objective of the purposed research work

1. Collection, authentication, processing and drying leaf extract of *Sphaeranthus indicus*
2. Cleaning, drying and grinding leaf extract of *Sphaeranthus indicus*
3. Preparation of hydro alcoholic leaf extract by using cold percolation method.
4. Preliminary qualitative Phytochemical analysis to identify chemical compound.

5. Investigation of Evaluation of Anti Hyperlipidemic Activity of *Sphaeranthus Indicus* Leaf extracts On Dexamethasone-Induced Hyper Lipidemia in Rats.
6. From the studies Phytochemical analysis, Anti Hyperlipidemic and Antioxidant activity performed under

The studies mentioned above utilized phytochemical and pharmacological evaluations to assess the therapeutic properties of the plant, particularly its potential for reducing lipid levels, thereby demonstrating its anti-hyperlipidemic effects.

Plant profile

Detailed Academic Profile of *Sphaeranthus indicus* L.

Sphaeranthus indicus, commonly known as **East Indian Globe Thistle**, **Mundi**, or **Gorakhmundi**, is a medicinal herb belonging to the family **Asteraceae**. Native to the tropical and subtropical regions of Asia, *S. indicus* is a widely recognized plant in traditional medicinal systems, particularly Ayurveda, Siddha, and Unani. Known for its versatile therapeutic properties, it has been used to treat various ailments such as skin disorders, inflammation, respiratory issues, and gastrointestinal problems. The plant's unique spherical flower heads and bioactive compounds make it a subject of increasing interest in modern pharmacological research.

Taxonomy and Classification

- **Kingdom**: Plantae
- **Clade**: Angiosperms
- **Order**: Asterales
- **Family**: Asteraceae
- **Genus**: *Sphaeranthus*
- **Species**: *Sphaeranthus indicus*

The genus *Sphaeranthus* includes several species, but *S. indicus* is the most studied and widely utilized due to its medicinal importance and availability in diverse habitats.

Morphological Characteristics

Growth Habit

Sphaeranthus indicus is an annual, aromatic herb that grows up to 15–60 cm in height. It is often found in wet or marshy areas and is known for its bushy appearance.

Leaves

- **Arrangement**: Opposite.
- **Shape**: Oblong or ovate with serrated margins.
- **Size**: 5–10 cm in length and 2–5 cm in width.
- **Texture**: Smooth to slightly hairy, depending on environmental conditions.
- **Color**: Bright green.

The leaves are aromatic and are often used in traditional medicine for their anti-inflammatory and analgesic properties.

Flowers

- **Inflorescence**: Globose, compact flower heads (capitula) with sessile florets.
- **Color**: Purple to pink.
- **Size**: Approximately 1–2 cm in diameter.
- **Fragrance**: Mildly aromatic, attracting pollinators like bees.
- **Blooming Period**: Year-round, with peak flowering during the rainy season.

Fruits

- **Type**: Achenes (dry, one-seeded fruit).
- **Shape**: Oval and flattened, with longitudinal ridges.
- **Size**: Small, around 1–2 mm in length.

Roots

The plant has a fibrous root system, which anchors it in wet soils and facilitates nutrient uptake.

Traditional and Modern Medicinal Uses

Traditional Medicine

Sphaeranthus indicus is widely used in Ayurveda, Siddha, and Unani for its multifaceted therapeutic properties. Common applications include:

1. **Skin Disorders**:
   - The paste of the flowers or leaves is applied to treat eczema, psoriasis, and other skin inflammations.
2. **Respiratory Issues**:
   - Decoctions are used to relieve asthma, cough, and bronchitis.
3. **Digestive Disorders**:
   - The plant is used to treat indigestion, flatulence, and diarrhea.
4. **Fever and Infections**:
   - Known for its antipyretic and antimicrobial properties, it is used to combat fevers and infections.

Sphaeranthus indicus is a versatile medicinal herb with immense therapeutic, ecological, and economic value. Its extensive use in traditional medicine, coupled with its growing applications in modern pharmacology, underscores its importance as a valuable natural resource. However, sustainable harvesting, cultivation, and further research are essential to ensure its availability for future generations. As a symbol of resilience and adaptability, *Sphaeranthus indicus* continues to serve humanity as both a healing herb and an ecological asset.

Supplies and techniques

Collection and Extraction of Medicinal Plant

Sphaeranthus indicus was collected in bulk from various locations. The plant material was shade-dried for 15 days and ground into a coarse powder using a pulverizer. Extraction was carried out using a 30:70 hydroalcoholic solvent (water:ethanol) via the cold percolation method. The extract was vacuum-dried, stored in a desiccator, and later refrigerated until use.

Phytochemical Constituent Identification

2.1 Preliminary Phytochemical Screening

Qualitative phytochemical analysis was conducted on the methanolic extract of Sphaeranthus indicus flowers using standard protocols described in Trease and Evans (1958) and Practical Pharmacognosy (Kokate, 2000).

Phytochemical Tests

Glycosides and Carbohydrates

Molisch Test: 1% alcoholic naphthol and concentrated sulfuric acid were added to the filtrate. A brown ring at the junction indicated carbohydrates.

Fehling's Test: Fehling's A and B reagents were added and boiled. A brick-red precipitate confirmed reducing sugars.

Legal Test: Hydrolysate was alkalinized and treated with pyridine and sodium nitroprusside. A pink-red color indicated glycosides.

Borntrager's Test: Ammonia was added to the chloroform layer of the hydrolysate. A pink layer confirmed glycosides.

Fixed Oils and Fats

Filter Paper Test: Oil stains on filter paper indicated fixed oils.

Saponification Test: Formation of soap after heating with alcoholic KOH confirmed the presence of fixed oils.

Amino Acids and Proteins

Millon's Test: A red coloration confirmed proteins.

Biuret Test: A violet color indicated proteins.

Ninhydrine Test: A purple coloration confirmed free amino acids.

Saponins

A persistent 1 cm foam layer after shaking with water indicated the presence of saponins.

Phenolic Compounds and Tannins
Ferric Chloride Test: A violet coloration indicated phenolic compounds.
Lead Acetate and Sodium Chloride Tests: White precipitate confirmed tannins.

Phytosterols
Salkowski Test: A brown ring indicated phytosterols.
Libermann-Burchard Test: A bluish-green color confirmed their presence.

Alkaloids
The filtrate treated with the following reagents yielded characteristic precipitates:
Wagner's: Reddish-brown
Mayer's: Cream
Dragendorff's: Orange-brown
Hager's: Yellow

Gums and Mucilages
No swelling in alcohol precipitate indicated the absence of gums and mucilages.
Flavonoids
Yellow coloration with NaOH and H_2SO_4, and pink color in the Shinoda Test, confirmed flavonoids.

Chemicals and reagents
All laboratory-grade solvents and reagents were obtained from SD Fine Chemicals, India. Dexamethasone and gemfibrozil were purchased from Merck, India and Pharpharma Ltd.

Experimental Animals
Species: Male Albino Wistar Rats
Weight Range: 180–230 g
Housing Conditions: Temperature 21 ± 2 °C, Relative Humidity 55–60%, 12-hour light/dark cycle
Diet: Standard pellet feed and water ad libitum
Ethical Approval: Approved by the Institutional Animal Ethical Committee (IAEC) of the College of Pharmacy.

Acute Toxicity Study
The up-and-down method was used. Methanolic extract (2 g/kg) was administered orally to six rats (3 male and 3 female). Animals were monitored continuously for 3 hours and then observed daily for 10 days for signs of toxicity or mortality.

Experimental Design

Parameter	Details
Number of Animals	6
Sex	Male
Strain	Albino Wistar rats
Body Weight	180–230 g

Drug Administration
Hyperlipidemic Agent: Dexamethasone (10 mg/kg, subcutaneously)
Standard Drug: Gemfibrozil (10 mg/kg, orally)
Test Drug: Sphaeranthus indicus extract (200 mg/kg, orally)
Vehicle: 1% CMC (carboxymethyl cellulose)

Model: Dexamethasone-Induced Hyperlipidemia
Dexamethasone administration increases triglyceride production and VLDL secretion, resulting in elevated serum lipid levels and fatty liver development. This model mimics lipid metabolism disorders and is used to assess the antihyperlipidemic potential of plant extracts.

Antioxidant Activity
DPPH Free Radical Scavenging
The ability of the extract to neutralize DPPH radicals (a marker for lipid peroxidation inhibition) was assessed. Results were compared with BHT (Butylated Hydroxytoluene) as the standard antioxidant.

ABTS Assay

ABTS+ radical cation decolorization was used to determine antioxidant capacity. Percentage inhibition at 736 nm was calculated to compare with ascorbic acid. The presence of antioxidant compounds in the extract reduced ABTS+ generation, indicating radical scavenging activity.

RESULT AND DISCUSSION

Phytochemical screening

The phytochemical screening results revealed that the after which it was observed whether the alkaloids were present due to absence of turbidity formation. The colournot changed from violet to blue or green in some samples indicated the absence of steroids. An interface with a reddish-brown coloration was formed in the absence of carbohydrates as negative result. Red coloration identifies the presence of flavonoids (Shinado's test). A colour change was observed in the test tube, which indicated the presence of tannins.

Table 1: Phytochemical screening of Sphaeranthus indicus **extract**

S.No	Phytoconstituents	Presence
1	Tannins	-
2	Alkaloids	+
3	Steroids	-
4	Glycosides	-
5	Flavonoids	+
6	Carbohydrates	-
7	Saponins	+

Dexamethasone induced hyperlipidemia in rats

Rats treated with Extract showed decreased serum levels of total cholesterol, LDL and triglycerides, compared to control hyperlipidemic rats. The HDL level of extract treated groups was constantly decreased when compared to normal and control group of animals. Similar results were observed for the standard drug of gemfibrozil used as positive control which has more potent hypolipidemic activity when compared to control and 200mg/kg of extract shown equipotent hypolipidemic action. Observed HDL levels indicated that the 200mg/kg treated animals observed were increased dose when compared to standard drug.

Extract 200 mg/kg treated groups were showed decreased serum levels of VLDL, altherogenic index Phospholipids and free fatty acids were respectively, compared to control hyperlipidemic rats. Which are clarified in the

Table 2: Effect of Hydroalcoholic extracts of Sphaeranthus indicus **against Dexamethasone induced hyperlipidemia in rats**

Group	Dose	Total Cholesterol (mg/dl)	Total TG (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)
I	Control (Normal)	63.1±1.352	56.84 ±1.77	35.67 ±1.689	17.57 ±0.333
II	Dexamethasone (10 mg/kg) S.C	107.84±1.687	146.84±1.667	32.17±0.307	64.34 ±1.687
III	Dexamethasone (10 mg/kg) S.C+ gemfibrozil (10mg/kg) P.O	84.51±1.352	63.34±0.764	38.34±0.421	26.45±0.33
IV	Dexamethasone with Extract (200 mg/kg)	73.04±1.45	73.43±0.35	27.14±0.57	19.10±0.42

All the values were represented as mean ± SEM. All the data were statistically analyzed by one-way **ANOVA** followed by Dunnett's test and values P.

Effect of Hydroalcoholic extracts against Dexamethasone induced hyperlipidemia in rats

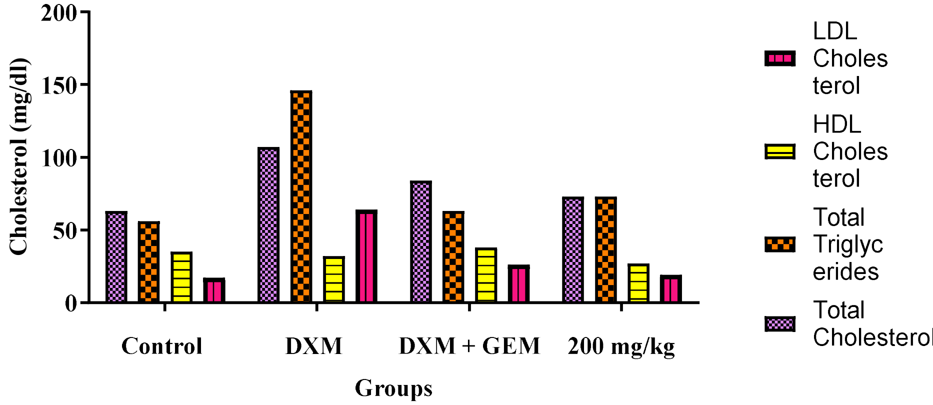

Fig 1: Dexamethasone induced hyperlipidemia in rats

Table 3: Effect of Hydroalcoholic extracts against Dexamethasone induced hyperlipidemia in rats

Group	Dose	VLDL Cholesterol (mg/dl)	Atherogenic index	Phospholipids (mg/dl)	Free fatty acids (mg/dl)
I	Control (Normal)	18.17 ±0.307	2.45	89.74 ±166	27.38 ±0396
II	Dexamethasone (10 mg/kg) S.C	36.74 ±1.542	4.76	143.2±2.983	28.2 ±0.152
III	Dexamethasone (10 mg/kg) S.C+ Gemfibrozil (10mg/kg) P.O	12.17 ±0.307	3.23	93.38 ±1.55	27.63 ±0.223
IV	Dexamethasone with Extract (200 mg/kg)	41.88±0.45	4.1	75.34±0.75	32.77±0.75

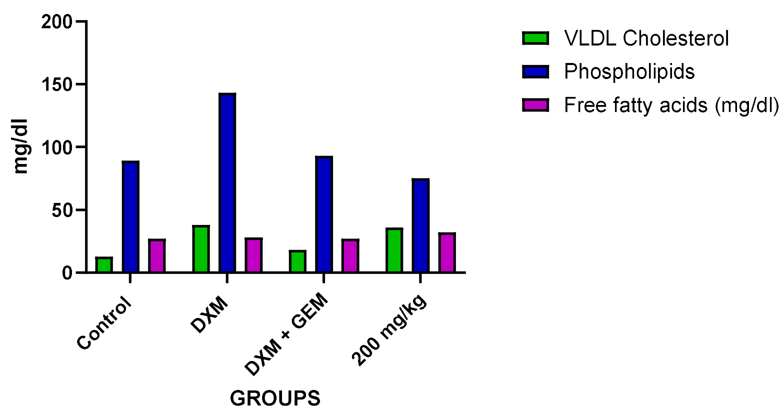

Fig 2: Dexamethasone induced hyperlipidemia in rats

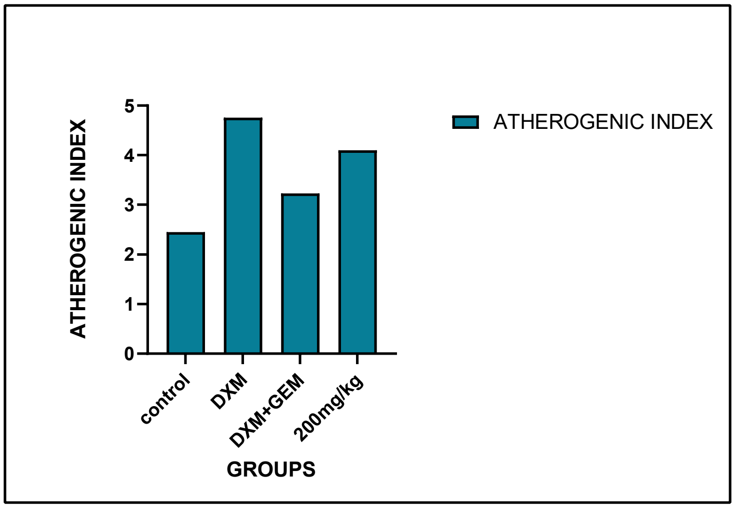

Fig 3: Atherogenic Index

***Invitro* antioxidant**

Results stated that the attendance of potentially antioxidant substances in Extract, an *in vitro* evaluation of DPPH free radical scavenging at different concentrations was performed. The 50% inhibitory concentration (IC_{50}) and the maximum activity in assay of DPPH free radical scavenging of Extract were equipotent action when compared to BHT as shown in Table. No.5 and Fig. No.13.

The results clarified that the turnout of potent antioxidant substances in Extract, an *in vitro* evaluation of ABTS free radical scavenging at different concentrations was performed. The 50% inhibitory concentration (IC_{50}) and the maximum activity in assay of DPPH free radical scavenging of Extract were equipotent action when compared to ascorbic acid as shown in Table. No.6 and Fig. No.14.

Table 4: *Invitro* antioxidant study by DPPH method

S.No	Concentration	Leaf Extract	Percentage Inhibition (%) of Standard
1.	50	58.53 ±0.89	33.58±0.37
2.	100	85.91±0.63	47.20±0.55
3.	150	92.34±0.32	42.09±0.20
4.	200	84.39±0.47	52.89±0.73
5.	250	86.99±0.91	60.41±0.59

Standard: Butylated hydroxyl toluene

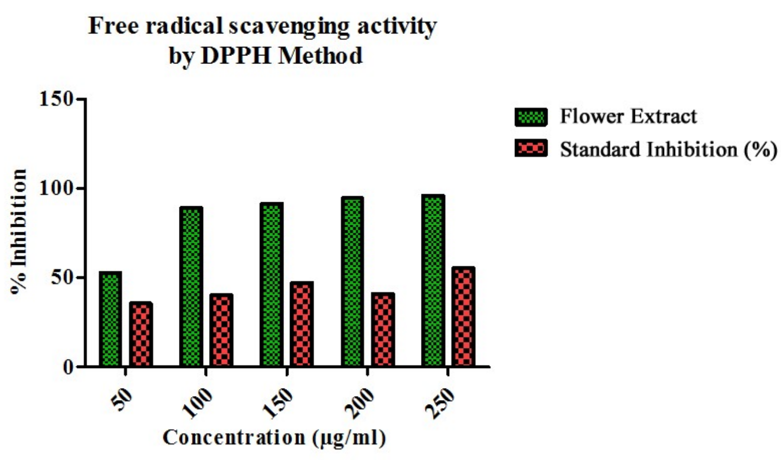

Fig 4: Antioxidant study by DPPHmethod

Table 5: Free radical scavenging activity by ABTS Method

S.No	Concentration	Flower Extract	Percentage Inhibition (%) of Standard
1.	200	0.61±0.56	0.43±0.05
2.	300	0.73±0.06	0.64±0.06
3.	400	0.79±0.95	0.68±0.09
4.	500	0.72±0.53	0.73±0.54
5.	600	1.39±0.28	0.94±0.08

Standard: Ascorbic acid

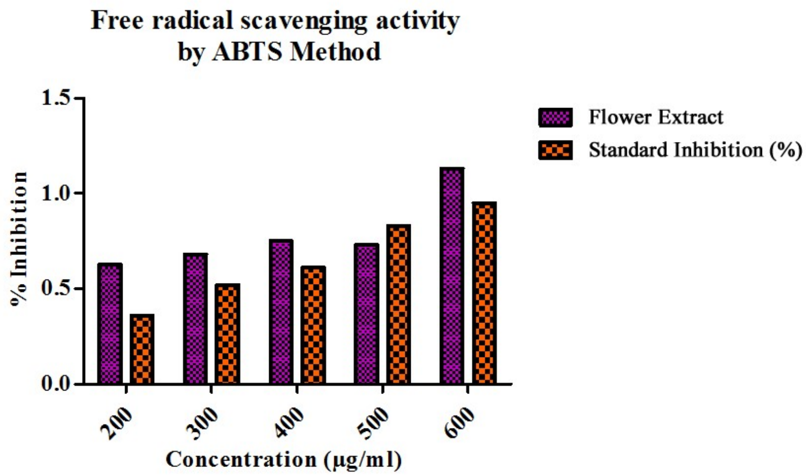

Fig 5: Antioxidant study by $ABTS^+$ method

CONCLUSION

This study confirms the antihyperlipidemic and antioxidant potential of Sphaeranthus indicus in dexamethasone-induced hyperlipidemic male Wistar rats. The extract significantly lowered serum triglycerides and cholesterol while exhibiting free radical scavenging activity, slightly outperforming BHT. Its lipid-lowering effects are attributed to phytoconstituents like flavonoids and saponins, which influence cholesterol metabolism and enzyme activity. The absence of adverse effects on liver and kidney functions highlights the extract's safety, supporting its promise as a natural, less toxic alternative to conventional drugs.

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