ISSN: 2278-2648

Intercontinental Journal of Pharmaceutical Investigations and Research (ICJPIR)

ICJPIR |Vol.13 | Issue 2 | Apr - Jun -2026

www.icjpir.com

DOI: https://doi.org/10.61096/icjpir.v13.iss2.2026.220-230

Antihyperlipidemic and Antioxidant Potential of Helicteres isora Fruit Extracts in High-Fat Diet-Induced Rats

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Published on: 25.05.2026

Published by: Futuristic Publications

Abstract: The present study was aimed at evaluating the antihyperlipidemic and antioxidant potential of hydroalcoholic fruit extracts of Helicteres isora in dexamethasone-induced hyperlipidemic rats. The fruits were collected, shade dried, powdered, and extracted using a hydroalcoholic solvent by cold percolation method. Preliminary phytochemical screening revealed the presence of alkaloids, steroids, flavonoids, and saponins in the extract. Antihyperlipidemic activity was evaluated in male Wistar rats by analyzing serum lipid parameters such as total cholesterol, triglycerides, HDL, LDL, VLDL, phospholipids, and free fatty acids. Treatment with Helicteres isora extract (200 mg/kg) significantly reduced total cholesterol, triglycerides, LDL, VLDL, phospholipids, and atherogenic index when compared to hyperlipidemic control rats. Antioxidant activity was assessed by DPPH and ABTS free radical scavenging assays, where the extract demonstrated significant free radical scavenging activity comparable to standard antioxidants such as butylated hydroxytoluene and ascorbic acid. The findings suggest that Helicteres isora fruit extract possesses significant antihyperlipidemic and antioxidant activities, which may be attributed to the presence of flavonoids and other phytoconstituents. The study supports the traditional use of Helicteres isora as a potential natural therapeutic agent for the management of hyperlipidemia and oxidative stress.

Keywords: Helicteres isora, Antihyperlipidemic activity, Antioxidant activity, Dexamethasone-induced hyperlipidemia, DPPH assay, ABTS assay, Flavonoids, Hydroalcoholic extract, Wistar rats, Oxidative stress.

1. INTRODUCTION

Helicteres isora is characterized as a substantial bush or a diminutive tree, showcasing hairy, egg-shaped foliage with toothed edges. It is categorized within the Sterculiaceae botanical group. The fruit manifests as a composite pod, exhibiting a spiraled configuration akin to a screw, culminating in a pointed extremity, thus justifying its designation as the Indian Screw Tree. The blossoms exhibit an orange-red pigmentation. This flora is dispersed throughout the Indian subcontinent, spanning from Punjab to Bengal, and from Jammu to the southern reaches of India. Commonly, this shrub or tree flourishes within the arid, seasonal forests of central and western India, ascending to altitudes of 1500 meters on mountainous inclines. It constitutes a prevalent botanical element in the central and western regions of India. Its presence extends to regions such as the Malay Peninsula, Java, and Australia. Various segments of the plant encompass a spectrum of significant antioxidants, including polyphenols and tannins, alongside being a commendable provider of vital nutrients (Gayathri P, et al 2010). These

aforementioned antioxidants and nutrients possess substantial substantiation regarding their dietary and therapeutic attributes.

In light of its nourishing and therapeutic worth, diverse components of the plant are integrated into traditional herbal remedies, exemplified by formulations like Gandharva Churna and Siddha Praneshwar Ras (Ahuja BS. 1965; Singh KK, et al 1985). Several native plant species utilized in traditional medicine show substantial therapeutic potential, yet confirmation of particular active ingredients through experimentation is absent. Among native medicinal plants, Helicteres isora stands out as a noteworthy plant, recognized for its considerable nutritional content and medicinal benefits. This shrub, native to tropical Southeast Asia, is widely cultivated across India. Various segments of this plant have long been employed in traditional Indian medical practices to alleviate a range of health conditions. Additionally, contemporary studies imply that H. isora contains numerous active substances like polyphenols, tannins, and alkaloids, which generate therapeutic effects. Additionally, H. isora is documented as a valuable origin of carbohydrates, proteins, fiber, calcium, phosphorus, and iron (Gayathri P, et al 2010). Another study involving extraction and characterization revealed some antioxidant agents such as ascorbic acid, flavonoids, and phenolics (cucurbitacin B and isocucurbitacin B) (Bean MF, et al 1985; Lee DH, et al 2010). Research also indicates that H. isora extracts demonstrate antibacterial, antidiabetic, and anticancer capabilities. Considering the extensive array of medicinal applications for H. isora, it seemed pertinent to succinctly review the current advancements and indicate potential future uses of its bioactive components in treating illnesses, including cancer (Dayal, R., et al 2015).

Helicteres isora

Taxonomical Classification

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Malvales

Family: Malvaceae

Genus: Helicteres

Species: *Helicteres isora*

SUPPLIES AND TECHNIQUES

Gathering, Classifying, and Extracting Medicinal Plants

This fruit was gathered in large quantities from different locations. After 15 days of shade drying, the collected fruits were ground into a coarse powder using a pulverizer. In order to

remove the ground plant material, a 30:70 ratio of hydro alcohol was used. The cold percolation method was used to perform the extraction. After being vacuum-dried, the extracts were kept in a desiccator before being placed in a refrigerator.

PHYTOCHEMICAL CONSTITUENT IDENTIFICATION

PHYTOCHEMICAL PRELIMINARY TESTS

Initial phytochemical analyses were conducted using the standard operating protocols outlined in Trease and Evans (1958)67 and the book Practical Pharmacognosy (Kokate, 2000)68. Below are the specifics of the same. To identify the different active ingredients, methanolic extracts of S *Helicteres isora* fruits were put through qualitative testing.

GLYCOSIDEANDCARBOHYDRATETEST

Four milliliters of distilled water were used to dissolve a tiny amount of extract, which was then filtered. The following tests were performed on the filtrate to look for the presence of glycosides and carbs.

The Molisch test

Two to three drops of 1% alcoholic naphthol were added to the filtrate, and two milliliters of concentrated sulfuric acid were applied along the test tube's walls. Carbohydrates are present when a brown ring appears where two liquids converge.

The Fehling test

One milliliter of Fehling's A and B was added to the filtrate, which was then boiled in a water bath. The presence of carbohydrates is shown by the reddish precipitate that was produced. To find out whether glycosides were present, a different quantity of the extract was hydrolyzed for a few hours in a water bath using diluted hydrochloric acid. The hydrolysate was then put through the following tests.

The legal test

The hydrolysate was turned alkaline with sodium hydroxide solution after 1 milliliter of pyridine and a few drops of sodium nitroprusside solution were added. The presence of glycosides is indicated by a pink to crimson appearance.

The Borntrager test

Chloroform was added to the hydrolysate, and the chloroform layer was removed. A diluted ammonia solution was added in an equal volume to this. When glycosides are present, the ammonia layer turns pink.

FIXED OIL AND FATS DETECTION

Test of filter paper

A tiny amount of extract was sandwiched between the filter sheets. The presence of fixed oils is indicated by the appearance of an oil stain on the paper.

Test for saponification

A drop of phenolphthalein and a few drops of 0.5 N alcoholic potassium hydroxide were added to a little amount of extract. For one to two hours, the mixture was heated in a water bath. The presence of fixed oils and fats is indicated by the formation of soap.

Identification of Free Amino Acids and Proteins

The following tests were performed after a little amount of extract was dissolved in a few millilitersofwater.

The Millon test

Millon's reagent was applied to the extract that was previously made. The formation of red indicates the existence of free amino acids and proteins.

The Biuret test

An equal volume of 5% sodium hydroxide and 1% copper sulphate solution were added to the extract that had been made above. The production of a violet hue indicates the existence of free amino acids and proteins.

Testforninhydrine

The Ninhydrine reagent was applied to the extracts. The production of purple indicates the existence of free amino acids and proteins.

Identifying saponins

After diluting the extract with 20 milliliters of distilled water, it was shaken for fifteen minutes in a measuring cylinder. Saponins are evident in the creation of a 1 cm layer of foam.

Identification of Phenolic Compounds and Tannins

Each extract was taken in small amounts in water, and the following reagents were used to test for the presence of tannins and phenolic compounds.
Fivepercentferricchloridesandvioletcolor10%sodiumchloride-whiteprecipitate
Tenpercentleadacetatesolution-whiteprecipitate The results above demonstrate the existence of tannins and phenolic chemicals.

PHYTOSTEROL DETECTION

A small amount of each extract was separately dissolved in 5 milliliters of chloroform. To find out whether phytosterols were present, this chloroform solution was then put through the following procedures.

TheSalkowskitest

A few drops of strong sulfuric acid were added to 1 milliliter of the previously produced chloroform solution. The production of a brown hue indicates the existence of phytosterols.

BurchardtestbyLibermann

A few drops of strong sulfuric acid, a few drops of diluted acetic acid, and three milliliters of acetic anhydride were added to the previously produced chloroform solution. The presence of phytosterols is indicated by the appearance of a bluish green color.

ALKALOIDDETECTION

A tiny amount of each extract was treated independently with a few drops of diluted hydrochloric acid before being filtered. The following experiments were conducted using the filtrate.

Wagner's reagent:

reddish brown precipitate; Mayer's reagent: cream precipitate; Dragendorff's reagent: orange brown precipitate; and Hager's reagent: yellow precipitate

GUMS AND MUCILAGES DETECTION

Each extract was added in little amounts to 25 milliliters of pure alcohol while being constantly stirred, and the mixture was then filtered. After being air-dried, the precipitate's swelling

characteristics were investigated. The absence of gums and mucilages is indicated by the lack of swelling.

DETECTION OF FLAVONOIDS

1. A small amount of each extract was separately dissolved in sodium hydroxide in water. Flavonoids can be identified by their yellow appearance.

2. Concentrated sulfuric acid was added to the tiny amount of each extract. The acquired yellow-orange color indicates the presence of flavonoids.

3. **Shinoda test:** Alcohol was used to dissolve a little amount of each extract. Drop by drop, powerful hydrochloric acid was poured to the magnesium bits, and they were then heated. The presence of flavonoids is indicated by a pink appearance.

Chemicals

SD Fine Chemicals India Ltd. provided the laboratory-grade solvents used in the investigation. Gemfibrozil, dexamethasone, and all other analytical-grade substances utilized in this investigation were acquired from Merck, India, and Pharpharma Ltd.

Animals

The male albino wistar rats utilized in the study weighed between 180 and 230 grams. They were kept in a typical laboratory setting with a relative humidity of 55–60% and room temperature of 21ºC±2ºC.They were given a regular pellet diet and unlimited access to water. The College of Pharmacy's Institutional Animal Ethical Committee (IAEC) gave its approval to the study protocol.

Research on acute toxicity

The up-and-down method was used to assess the acute toxicity of *Helicteres isora* in rats.Three female and three male rats weighing between 150 and 200 g were given methanolic extract of *Helicteres isora* orally by gavage at a starting dose of 2 g/kg. After the first three hours of dosage, the animals were continuously monitored for toxic symptoms. After twenty-four hours, the number of survivors was recorded, and the animals were kept for an additional ten days, during which time daily observations were taken.

EXPERIMENTAL DESIGN

ANIMAL

Total number of animal	: 6
Sex	: Male
Strain	: Albino wistar rats
Body weight	: 180-230 gm

Administration of Surfactants

for hyperlipidemia Agent : dexamethasone
Administration route : subcutaneous

Administration of Drugs

Vehicle: 1% CMC
The oral route of administration
The recommended dosage of gemfibrozil is 10 mg/kg.
The extract is 200 mg/kg.

Model of hyperlipidemia produced by dexamethasone

Although it differs from species to species, the elevation of glucocorticoid hormone levels causes the plasma lipid concentration. When rats are injected with glucocorticoids, their livers produce less triacylglycerol, which can result in the buildup of fatty liver. VLDL secretion may rise as a result of TG production stimulation. Injecting rats with dexamethasone for a few days has been shown to increase VLDL secretion. Hyperlipidemia results from an imbalance in lipid metabolism brought on by an increase in TG levels. Likewise, four days of dexamethasone therapy in neonatal rats resulted in a general rise in serum lipid levels.To assess the antihyperlipedemia impact of Sphaeranthus indicus extract at a dose level of 200 mg/kg, six mice were grouped. The information for the standard, hyperlipedimic-induced, and control groups was gathered from an earlier Indian journal review.

DPPH technique for scavenging free radicals

One of the common free radicals used to test a plant extract's initial capacity to scavenge radicals is DPPH. Lipid peroxidation inhibition is linked to DPPH radical scavenging. DPPH is typically employed as a material to assess antioxidant activity.51–56.

Activity that scavenges free radicals The ABTS MethodThe decolorization of ABTS+ is determined by calculating the percentage inhibition of absorbance at 736 nm, which represents the reduction of the radical cation. The ABTS+ chromophore was incubated through the process to produce ABTS+. Certain chemical compounds found in Sphaeranthus indicus extracts may inhibit potassium persulfate action, which would lower ABTS+ generation.

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 colour not 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 *Helicteres isora* 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 were 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 have 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. Which are justified in

the Table.No.3 & Fig.No:10. 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.3 & Fig 2.

Table 2: Effect of Hydroalcoholic extracts of *Helicteres isora* 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)	45.1±1.356	52.64 ±1.77	45.67 ±1.685	18.58±0.333
II	Dexamethasone (10 mg/kg) S.C	115.84±1.486	157.74±1.667	36.17±0.336	68.74 ±1.687
III	Dexamethasone (10 mg/kg) S.C+ gemfibrozil (10mg/kg) P.O	67.51±1.647	58.84±0.764	39.34±0.475	28.95±0.73
IV	Dexamethasone with Extract (200 mg/kg)	54.04±1.37	78.23±0.35	19.14±0.46	25.60±0.82

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.

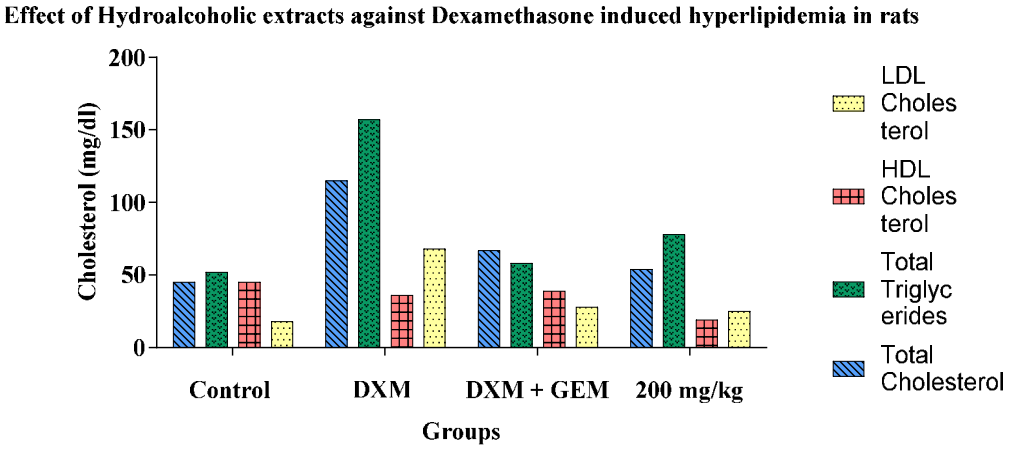

Fig 1: Dexamethasone induced hyperlipidemia in rats

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)	25.17 ±0.356	4.45	79.74 ±1.66	29.38 ±0396
II	Dexamethasone (10 mg/kg) S.C	38.74 ±1.587	8.76	135.2±2.983	23.2 ±0.152

III	Dexamethasone (10 mg/kg) S.C+ Gemfibrozil (10mg/kg) P.O	18.17 ±0.394	4.23	95.38 ±1.55	30.63 ±0.223
IV	Dexamethasone with Extract (200 mg/kg)	39.88±0.75	5.1	78.34±0.75	31.77±0.75

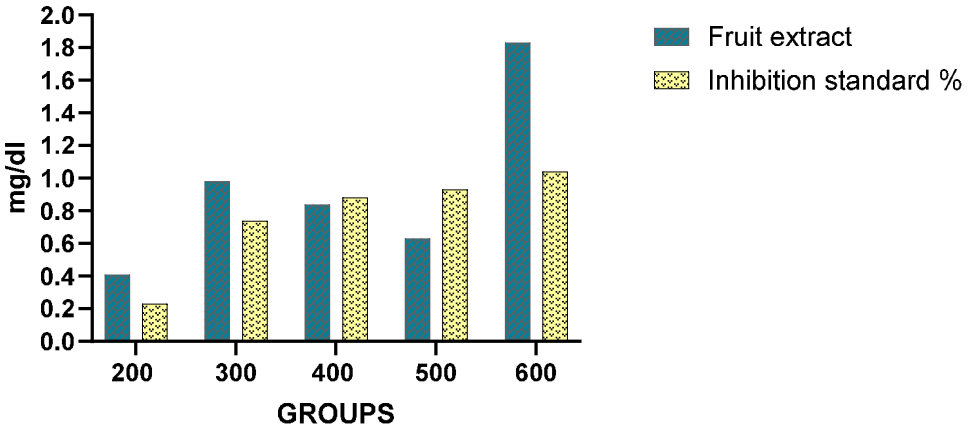

Fig 2: Dexamethasone induced hyperlipidemia in rats

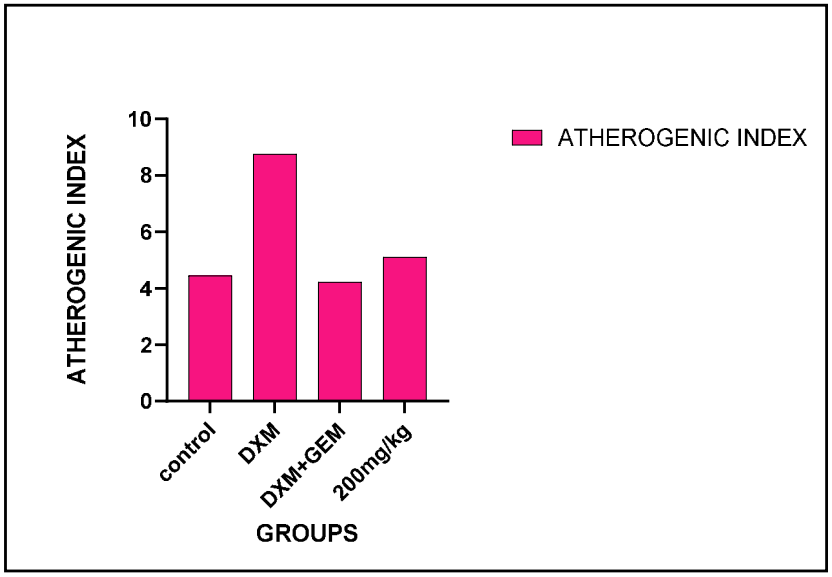

Fig 3: Atherogenic Index

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 & 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 & Fig. No.14.

Table 4: *Invitro* antioxidant study by DPPH method

S.No	Concentration	Fruit Extract	Percentage Inhibition (%) of Standard
1.	50	47.53 ±0.56	23.58±0.37
2.	100	84.91±0.74	57.20±0.55
3.	150	94.34±0.38	62.09±0.20
4.	200	76.39±0.44	72.89±0.73
5.	250	92.99±0.98	76.41±0.59

Standard : Butylated hydroxyl toluene

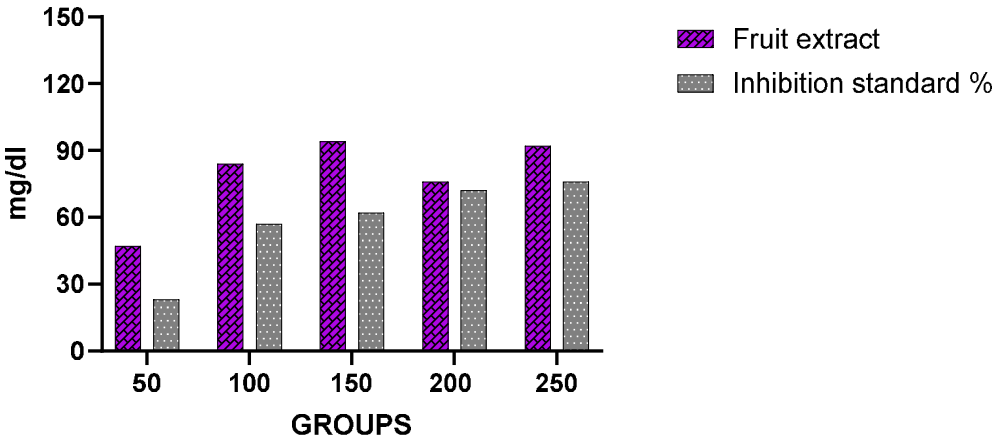

Fig 4: Antioxidant study by DPPH method

Table 5: Free radical scavenging activity by ABTS Method

S.No	Concentration	Flower Extract	Percentage Inhibition (%) of Standard
	200	0.41±0.56	0.23±0.05
	300	0.93±0.06	0.74±0.06
	400	0.84±0.95	0.88±0.09
	500	0.63±0.53	0.93±0.54
	600	1.84±0.28	1.04±0.08

Standard: Ascorbic acid

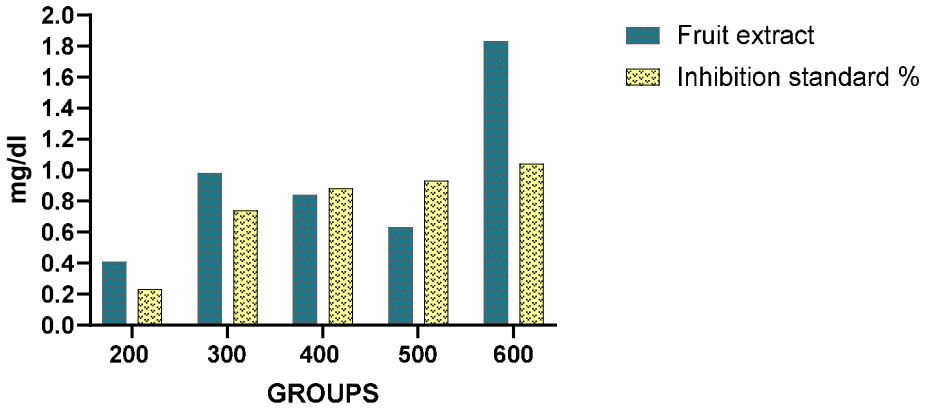

Fig 5: Antioxidant study by ABTS^{+} method

CONCLUSION

The current research focuses on Antihyperlipidemic activity in dexamethasone-induced hyperlipidemia in male Wistar rats, with the extracts of *Helicteres isora*. The antihyperlipidemic potential of the *Helicteres isora* has been planned to be assessed for its activity against controlling the lipid profile of the animal selected models. Thus the results of the present investigation clearly indicated that the selected medicinal plants possess good antihyerlipidemic activity in dexamethasone induced hyperlipidemic rats and led to the possessing of antihyperlipidemic and selected species activities. The results found are encouraging for further studies on the selected plants and to identify the bioactive compounds.

Oxidative balance in the body was regulated by endogenous and exogenous mechanisms, in which surplus of free radicals connected to many diseases. By regulation of the excess of oxidative molecules includes especially exogenous intake of antioxidants, which are largely found in natural sources. The chemical composition of these plants has shown that same classes of polyphenols may present which are exert such function such as flavonoids. The capacity of Extract of DPPH free radicals scavenging was intermediary among standard antioxidants just about higher than that of BHT.

The significance of novel products in the action and avoidance of dyslipidemias becomes necessary to reduce the mortality and morbidity due to cardiovascular complications. In addition, the search for less toxic drugs has augmented the interest of the scientific community for natural products. The Extract showed to able to manage hyperlipidemia induced by high-fructose diet, reducing serum levels of total cholesterol and triglycerides, without signs may be indicated change in hepatic and renal function, suggesting that Extract is safe in the evaluated conditions. The hypolipidemic activity of natural products can be correlated to the presence of flavonoids due to their properties of inhibiting cholesterol biosynthesis and absorption and modifying the activity of lipogenic and lipolytic enzymes, leading to reduced lipid metabolism, as observed in hyperlipidemic rats treated with Extract which showed important reduction in the levels of total cholesterol and triglycerides. Other molecules clever to decrease the serum level of cholesterol are saponins, also present in Extract. It is very interesting that Extract was able to reduce both serum level of cholesterol and total triglycerides.

In conclusion, our results showed that *Helicteres isora* reduce oxidative stress by free radical scavenging and protect adjacent to lipid peroxidation and also able to manage hyperlipidemia by decreasing serum level of cholesterol and triglycerides, similarly to standard drugs

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