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Pharmacodynamic findings for the usefulness of *Luffa cylindrica* (L.) leaves in atherosclerosis therapy with supporting antioxidant potential

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Abstract

Background: *Luffa cylindrica* (L.) is a commonly used vegetable in different parts of Asia. Its fruits are generally used as a vegetable, but pharmacological activities of the leaves were unrevealed. The study evaluated the antihyperlipidemic activity and in vitro antioxidant potential of methanolic extract of *Luffa cylindrica* (L.) leaves (MELCL). The antihyperlipidemic potential was investigated in Triton X-100-induced hyperlipidemic rats. Animals were pre-treated with Triton X-100 (400 mg/kg). The Triton X-100-treated animals were then treated with MELCL at the doses of 100 and 200 mg/kg using 5% CMC, as a vehicle, per oral (p.o) for 7 days. Antioxidant activity was studied by examining the DPPH and hydrogen peroxide radical scavenging potential of the extract.

Results: The plasma sample of rats was analyzed, and it was found that MELCL shows significant ($p < 0.05$) antihyperlipidemic activity at 200 mg/kg of MELCL. Serum analysis showed a marked reduction in the level of multiple biochemicals like total cholesterol (TC) (85.48 ± 3.230 mg/dl), triglycerides (TG) (74.62 ± 8.764 mg/dl), low-density lipoproteins (LDL) (31.97 ± 3.475 mg/dl), very low-density lipoproteins (VLDL) (14.92 ± 1.635 mg/dl), and an increase in the level of high-density lipoproteins (HDL) (40.58 ± 1.625 mg/dl). MELCL also showed significant scavenging of DPPH radical (46.66 ± 0.002%) at concentration and hydrogen peroxide radical (47.55 ± 0.001%) at 100 µg/ml.

Conclusion: Quantitative results of the study showed that MELCL has considerable antihyperlipidemic and antioxidant potential and could be the option for the treatment of atherosclerosis.

Keywords: *Luffa cylindrica* (L.), Triton X-100, Antihyperlipidemic, Atherosclerosis, Antioxidant, Lipoproteins

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Background

Hyperlipidemia is characterized by an increase in the level of plasma lipids, including cholesterol, cholesterol esters, triglycerides, lipoproteins, and phospholipids [1]. An elevation in the level of plasma lipids may occur due to primary hereditary disease, diet, or drugs. Hyperlipidemia is the prime reason for atherosclerosis and related conditions, such as ischemic cerebrovascular disease, coronary heart disease (CHD), and peripheral vascular disease. Several chemical categories of medications are used to treat hyperlipidemia [2]. Although several efficient lipid-lowering medications from the synthetic origin are available, their effectiveness is specific for certain lipoprotein disorders, along with the limitation of associated adverse effects. It is an important need of the era to find alternatives from natural origin with less associated side effects and efficient therapeutic efficacy [3]. A close relationship between oxidative stress and lifestyle-related diseases such as atherosclerosis hypertension, diabetes mellitus, ischemic diseases, and malignancies has been reported [4].

The role of antioxidants is significant to protect the body from different diseases and disorder caused due to free radical-induced oxidative stress [5]. The formation of reactive oxygen species (ROS) and free radicals constantly occur in the human body, and the defense system of the body continuously works to remove it out [6]. Natural antioxidants are gaining much interest in recent years among researchers. Phytochemicals like polyphenols are important to demonstrate antioxidant potential as they prevent oxidative damage [7].

Luffa cylindrica (L.) is a commonly found vegetable in most Asian countries. *Luffa cylindrica* (L.) is a frequently used vegetable in the tropical and subtropical region from the family Cucurbitaceae, commonly called sponge gourd, loofa, and vegetable sponge [8]. According to the previous phytochemical investigation, the leaves contain flavonoids, saponins, and triterpenes. Leaf also contains cardiac glycosides, tannins, whereas in fruits, ascorbic acid, anthocyanins, flavonoids, triterpenoid, and saponins are present [9]. The flowers are rich in flavonoids and carotenoids. The plant is immune-stimulant, used in the hypersensitive reaction, and an oxytocic. Seeds have a bronchodilator effect [10]. Fruits are antifungal and antibacterial. Leaves of the plants were found to be useful in the treatment of the decayed teeth, amenorrhea, and parasitic infections. The leaves and flowers also show antiemetic activity [11]. The plant also has purgative property and is employed for edema, nephritis, bronchitis, and respiratory organ complaints [12]. Leaves of *Luffa cylindrica* (L.) is traditionally used for weight loss, but the experimental demonstration and evidence of this pharmacological activity are required hence, the present work aimed to evaluate the antioxidant and antihyperlipidemic activity of methanolic extract of leaves of *Luffa cylindrica* (L.).

Methods

Collection of plant and procurement of chemicals

The leaves of *Luffa cylindrica* Linn. (Cucurbitaceae) were collected in December 2015 from Ambajogai, Maharashtra, India. The plant was authenticated from Postgraduate Botany Department, N.E.S. Science College, Nanded, as *Luffa cylindrica* (L.) (Cucurbitaceae) with a voucher specimen no: S-1/21/02/15. Required chemicals were brought from a local market manufactured at the Qualigens Fine Chemicals, Mumbai.

Experimental animals

Albino Wistar rats of either sex or weight between 150 and 200 g were used from the animal house of the Nanded College of Pharmacy, Nanded, Maharashtra. Animals were housed in a group of four in separate cages and maintained at controlled conditions of temperature (28 ± 2 °C). The standard pellet food and purified drinking water were provided with free access. After completion of the experimental procedure, all the animals were sacrificed with euthanasia using ether up to their normal death using inhalation anesthetic.

Table 1 Results of phytochemical screening of Methanolic Extract of Luffa *cylindrica* (L.) leaves

Phytochemical tests	Result
A) Carbohydrates	
1)Molisch's Test	+ve
2) Benedict Test	+ve
B) Alkaloids	
1) Mayer's Test	-ve
2) Wagner's Test	-ve
C) Glycosides	
1) Modified Borntrager's Test	+ve
2) Legal's Test:	+ve
D) Flavonoids	
1) Shinoda Test	+ve
2) Lead acetate Test	+ve
E) Phytosterols	
1) Salkowski's Test	+ve
2) LibermannBurchard's Test	+ve
F) Tannins	
1)Gelatin Test	+ve
G) Saponin+ve	

+ve indicates presence and **– ve** indicates absence

Preparation of extract

The leaves of *Luffa cylindrica* (L.) were thoroughly washed with running tap water 2–3 times. After the shade drying, coarsely powdered, the material was used for extraction. Five hundred grams of the material was extracted using solvents with different polarity, namely methanol, ethanol, and chloroform by soxhlet extraction. Extraction was carried out in 48 cycles, and every cycle was of 1 h each so the total extraction was carried for 48 h. The extract was filtered and dried on a water bath and stored at room temperature. The methanolic extract was chosen for the animal study due to the presence of flavonoids, polyphenols, tannin, and saponins [13].

Phytochemical screening

Phytochemical screening was carried out to determine the presence of different phytochemicals. The standard procedures were performed for the determination of different phytochemicals. After performing the test with specific reagents, the inference was given by visual observation of color change or precipitate formation [14].

Acute toxicity studies

Acute toxicity of methanolic extract of *Luffa cylindrica* (L.) leaves was determined using male Swiss albino rats as per OECD guideline 423. After overnight fasting, the weight of the individual rat was recorded before the study. Animals were divided randomly into four treatment groups; each group consisting of three rats and each treatment group receives orally the methanolic extract of *Luffa cylindrica* (L.) leaves in a dose of 2000 mg/kg. Close observation of animals was done for 4 h after the administration of the extract, and further observation was done up to 14 days to notice if any change occurs in general behavior and/or other physical activities [15].

Antihyperlipidemicactivitystudy of *Luffa cylindrica* (L.) leaf extract on Triton X-100-induced rats

The Wistar rats were randomly divided into 5 groups, and each group was comprised of 6 rats. A standard pellet diet was given to the first group along with water and 5% CMC was administered orally. The animals from the II, III, IV, and V groups were intraperitoneally injected with 10% aqueous solution of Triton X-100 (400 mg/kg body weight). Five percent CMC (p.o) was given to the second group for 7 days after 72 h of administration of Triton X-100 injection. MELCL was daily administered to the third and fourth group with the doses of 100 and 200 mg/kg using 5% CMC, as a vehicle, per oral (p.o) for 7 days, after inducing hyperlipidemia.

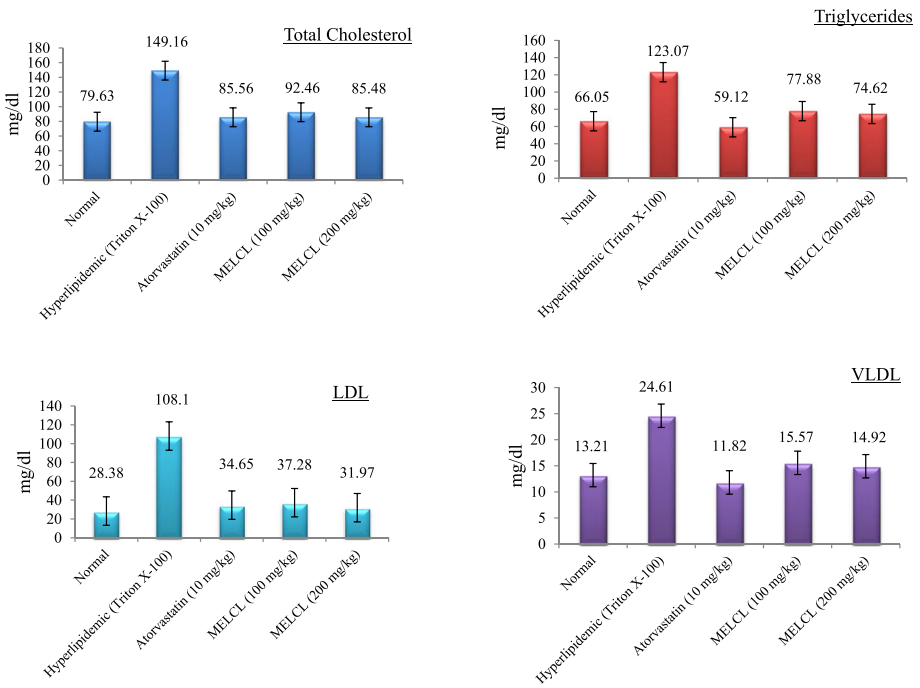

Fig. 1 Biochemical estimation of different groups for the level of Total Cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL). MELCL: Methanolic extract of *Luffa cylindrica* (L.) leaves

Atorvastatin was administered to the fifth group with a dose of 10 mg/kg, p.o for 7 days. Before blood sampling for biochemical estimation, the food was withdrawn 10 h prior, and on the 8th-day, biochemical estimation was performed [16].

Biochemical estimation

The blood samples were collected on the 8th day for all the groups by the retro-orbital puncture after sacrificing the animal with inhalation ether euthanasia. After the clotting of blood, the centrifugation was carried out at 3000 rpm for 10 min. The serum sample was subjected to biochemical analysis for the determination of serum levels of triglycerides, total cholesterol, low-density lipoprotein cholesterol, very-low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol. The determination of biochemical parameters was carried out as per the standard procedure provided by the manufacturer in the instruction manual of the auto analyzer kit (Hitachi 704 analyzer) [17].

Antioxidant potential of *Luffa cylindrica* (L.) leaf extract

DPPH (2,2-diphenyl, 1,1-picrylhydrazyl) radical scavenging activity

The solution of DPPH was prepared by dissolving 1.97 mg of DPPH in 50 ml methanol. The sample solution of extract was prepared by dilution method by diluting the extract up to 100 μg/ml. 0.2 ml of the sample solution of MELCL was taken and the standard (ascorbic acid), at a concentration of 50, 100, 150, and 200 μg/ml was added. One milligram of 0.1 mM freshly prepared DPPH radical solution was added. After vigorous shaking, the reaction mixture is allowed to stand for 30 min at room temperature. The control mixture contains all the reagents except the extract and is considered as a blank. An ultraviolet-visible spectrophotometer (UV-1800, SHIMADZU, Japan) was used to measure the absorbance of all reaction mixtures at 517 nm. The DPPH radical scavenging potential (%) of the sample was calculated with the following formula:

$$\%\text{Inhibition} = A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}} \times 100 \quad (1)$$

where A_{blank} is the absorbance of ascorbic acid and A_{sample} is the absorbance of extract. All tests were performed in duplicate [18].

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide solution (40 mmol/L) was prepared using phosphate buffer (pH 7.4) as a vehicle. The concentration of hydrogen peroxide was determined at 230 nm using a UV-visible spectrophotometer (UV-1800, SHIMADZU, Japan). Test samples (50, 100, 150, 200, and 250 μg) were added to a hydrogen peroxide solution (0.6 ml, 40 mmol/L). Absorbance was detected at 230 nm and was determined after 10 min against a blank solution. The scavenging of hydrogen peroxide by the sample and ascorbic acid was calculated using the same equation as above [19].

Statistical analysis

The statistical analysis of data was carried out using InStat-GraphPad (3.0). The values are expressed as mean ± SEM (n = 6) along with ANOVA followed by Dunnett test.

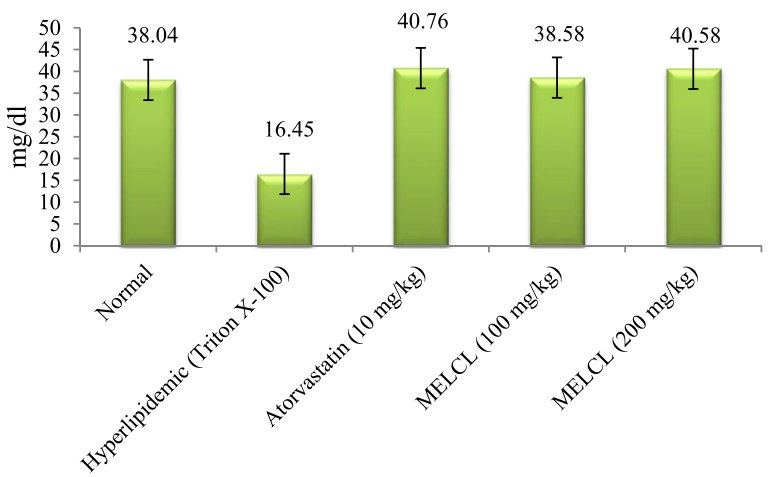

Fig. 2 Biochemical estimation of different groups for the level of High Density Lipoprotein (HDL). MELCL: Methanolic extract of *Luffa cylindrica* (L.) leaves

Table 2 Effect of methanolic extract of Luffa cylindrica (L.) leaves on Biochemical Parameters in wistar rats

Group	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL(mg/dl)
Normal	79.63 ± 2.715	66.05 ± 1.069	38.04 ± 1.960	13.21 ± 0.213	28.38 ± 2.319
Hyperlipidemic (Triton X-100)	149.16 ± 4.467	123.07 ± 5.421	16.45 ± 1.416	24.61 ± 1.084	108.1 ± 16.779
Atorvastatin (10mg/kg)	85.56 ± 6.128**	59.12±7.242***	40.76±1.404***	11.82±1.449**	34.65±4.642***
MELCL (100mg/kg)	92.46 ± 6.032**	77.88 ± 8.178**	38.58±1.767***	15.57±1.759**	37.28±7.254**
MELCL (200mg/kg)	85.48±3.230***	74.62 ± 8.764**	40.58±1.625***	14.92±1.635**	31.97±3.475***

Values are expressed as mean ± S.E.M; n=6, *P<0.05, **P<0.01, ***P<0.001 when group hyperlipidemic control compared with normal and group atorvastatin, MELCL(100mg/kg) and MELCL (200mg/kg) were compared with hyperlipidemic control considered for significance, (ANOVA followed by Dunnett's test)
MELCL Methanolic extract of *Luffa cylindrica* (L.) leaves, *TC* Total Cholesterol, *TG* Triglycerides, *LDL* Low-Density Lipoproteins, *VLDL* Very Low-Density Lipoproteins, *HDL* High-Density Lipoproteins

Results

The phytochemical screening showed that leaves extracts of *Luffa cylindrica* (L.) had different secondary metabolites such as glycosides, tannins, flavonoids, and saponins (Table 1)

Acute toxicity study

After administration of MELCL 2000 mg/kg to the rats, no behavioral changes were observed and adverse effects were found which indicated the safety of phytochemical extract without any toxicity consequences. Normally, the animals were healthy without any skin rashes or physical infirmity. A similar physiological condition was observed even after the toxicity study. Diet consumption by the animals was normal after the acute toxicity study.

Antihyperlipidemic activity of *Luffa cylindrica* (L.)

The TC, LDL, VLDL, and TG levels were significantly higher in Triton X-100-injected animals as compared to the controlled group. The present study showed that all Triton X-100-induced rats showed hyperlipidemia as there were increased levels of serum cholesterol, triglyceride, LDL, and decreased HDL level (Figs. 1 and 2). It was observed that increased total cholesterol, total lipids, and triglycerides levels were considerably reduced by the treatment of 100 and 200 mg/kg of MELCL. Reduction in TC at 200 mg /kg of MELCL causes a similar reduction as that of atorvastatin; however, atorvastatin causes more depletion. Similarly, that atorvastatin HDL levels also increased from 16.45 to 40.58 and a greater reduction in LDL occurs with 200 mg/kg of MELCL which is comparable to that of atorvastatin. The methanolic extract of *Luffa cylindrica* (L.) leaves demonstrated antihyperlipidemic activity in a dose-dependent manner (Table 2).

Atherogenic index analysis

The ratio of LDL-C (low-density lipoprotein cholesterol) and HDL-C (high-density lipoprotein cholesterol) is known as the atherogenic index, which is presented in Fig. 3. estimated for all the groups of animals. The ratios were significantly higher in TritonX-100-treated rats as

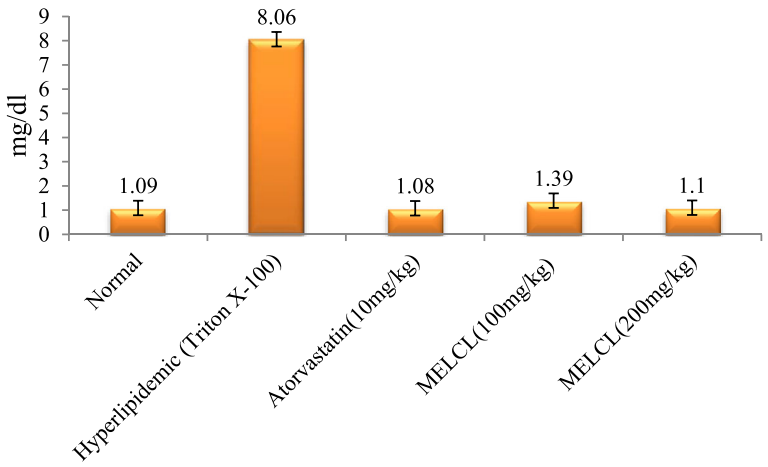

Fig. 3 Atherogenic index analysis for different groups. MELCL: Methanolic extract of *Luffa cylindrica* (L.) leaves

Table 3 Effect of MELCL on atherogenic index and percentage protection of various groups

Group	Atherogenic Index	% Protection
Normal	1.09 ± 0.017	-
Hyperlipidemic Control	8.06 ± 0.011	-
Atorvastatin(10mg/kg)	1.08 ± 0.014**	76.93
MELCL(100mg/kg)	1.39 ± 0.07*	73.62
MELCL(200mg/kg)	1.10 ± 0.017**	76.82

Values are expressed as mean ± S.E.M; n=6, *P<0.05, **P<0.01, ***P<0.
MELCL Methanolic extract of *Luffa cylindrica* (L.) leaves

compared to the normal group, and elevated ratios were found significantly reduced in groups of rats treated with MELCL and atorvastatin. Percentage protection provided by MELCL (100 and 200 mg/kg) was 73.62 and 76.82%, respectively, and 76.93% for atorvastatin-treated group (Table 3).

Antioxidant activity

DPPH radical scavenging activity

In the DPPH radical scavenging activity study, the antioxidants reduce DPPH radical to the yellow-colored diphenylpricrylhydrazine. The result of the DPPH-free radical scavenging ability of methanolic extract of *Luffa cylindrica* (L.) leaves is shown in Table 4 and compared with ascorbic acid (Fig. 4).

Hydrogen peroxide radical scavenging activity

MELCL exerted relatively the same hydrogen peroxide radical scavenging activity when compared with ascorbic acid (Table 5). Graphical representation of scavenging activity also reveals the comparative efficiency of MELC L (Fig. 4).

Discussion

The use of medicinal plants and herbs are the important alternatives for the treatment of numerous diseases and disorders due to increased efficiency and therapeutic activities of new plant-derived drugs and arising concerns about the side effects of existing medicinal therapy. Hyperlipidemia has been reported to be one of the major risk factors for cardiovascular diseases, including atherosclerosis and myocardial infarction [20]. The medications from synthetic origin available in the market show multiple side effects. One of the most common side effects of some antihyperlipidemic drugs, that decrease TC, is the reduction of the cardioprotective HDL-C. The search for new antihyperlipidemic agents with greater efficacy and few side effects is important [21].

In the present study, the Triton X-100 model was used as an acute model for the induction of hyperlipidemia in rats. Triton X-100 acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extrahepatic tissues, resulting in increased blood lipid concentration [22].

This study has shown the potential hypolipidemic and antioxidant effect of *Luffa cylindrica* (L.) on Triton X-100 treated albino Wistar rats. Treatment with Triton X-100 caused substantial elevation in TC, TG, LDL, and VLDL, which is indicative of the induction of hyperlipidemia. MELCL at the doses 100 and 200 mg/kg p.o. exerted a significant hypolipidemic activity [23]. Especially, MELCL at 200 mg/kg p.o. dose demonstrated a remarkable reduction in TC, TG, LDL, and VLDL. These effects are almost similar to that of atorvastatin, and even better reduction in VLDL was witnessed with MELCL at 200 mg/kg p.o. dose. HDL is considered as a protective lipoprotein that opposes the increase in TC and TG levels. Progression of hyperlipidemia is also indicated by the decrease in HDL level and that may further lead to coronary occlusion and increase the risk of CHD [24]. An increment in HDL to normal levels occurred with MELCL treatment. High HDL levels in the blood minimize the risk of cardiovascular diseases by reversing the transport of cholesterol and making cholesterol to return to the liver from the peripheral tissue promoting

Table 4 DPPH Scavenging Activity of MELCL

Sr. no	Concentration (µg/mL)	%Scavenging (Test)	%Scavenging (Standard)
1	10	19.68 ± 0.001	26.42 ± 0.005
2	20	19.29 ± 0.002	36.7 ± 0.006
3	40	33.16 ± 0.004	39.89 ± 0.003
4	60	34.71 ± 0.004	41.96 ± 0.006
5	80	39.89 ± 0.004	45.07 ± 0.006
6	100	46.66 ± 0.002	49.22 ± 0.005

Values are expressed as mean ± S.E.M; n=6
DPPH 2,2-Diphenyl 1,1-Picrylhydrazyl
MELCL Methanolic extract of *Luffa cylindrica* (L.) leaves

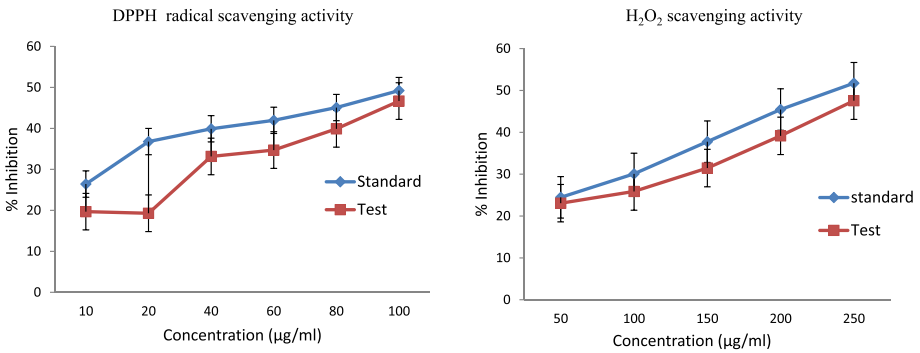

Fig. 4 MELCL antioxidant activity analysis by DPPH and Hydrogen peroxide scavenging activity model. MELCL**:** Methanolic extract of *Luffa cylindrica* (L.) leaves. DPPH: 2,2-Diphenyl 1,1-Picrylhydrazyl

anti-atherogenic activity [25]. The atherogenic index has been reported to consider as a powerful indicator of the risk of heart disease. A similar reduction in the atherogenic index was observed at both 100 and 200 mg/kg p.o. doses. Also, the percentage of protection observed was comparable to that of atorvastatin. These effects are indicative of the strong protective action of MELCL against hyperlipidemia [26].

Oxidative stress is commonly associated with hyperlipidemia and other-related disorders. Inconsistent with the antihyperlipidemic action, MELCL also demonstrated a significant dose-dependent antioxidant activity which is comparable to ascorbic acid in DPPH radical and hydrogen peroxide radical assays [27]. The % inhibition of DPPH shown by the methanolic extract was found to be (46.66%) significant in comparison with the positive control of ascorbic acid (49.22%) at 100 µg/ml. In hydrogen peroxide scavenging activity, the increase in the concentration of MELCL (50–250 µg/ml) leads to corresponding increases in the scavenging rate (23.07–47.55%). On the other hand percentage inhibition shown by ascorbic acid was found to be (24.47–51.74%) at the concentration of 50–250 µg/ml. Therefore, MELCL shows a promising antioxidant potential.

These scavenging effects may be partly responsible for its protective action against hyperlipidemia.

Phytochemical compounds such as tannins, saponins, alkaloids, flavonoids, and terpenoids have been implicated in free radical scavenging, antioxidant, and hypolipidemic activity. The presence of the mentioned constituents in the extract may in part be responsible for the observed effects [28].

Conclusion

The findings of the current work demonstrate that the methanolic extract of *Luffa cylindrica* (L.) leaves can improve the serum lipid profile in rats by decreasing the serum levels of TC, TG, and LDL cholesterol and increasing the HDL cholesterol level, thus improving the atherogenic index. DPPH radical and hydrogen peroxide radical assays also revealed potential antioxidant activity. This significant medicinal potential provides an opportunity for exploration of the pharmacokinetic investigation after purification of extract. Formulation development and commercial adaptation are also possible after concerning the present investigation.

Abbreviations

MELCL: Methanolic extract of *Luffa cylindrica* (L.) leaves; TC: Total cholesterol; TG: Triglycerides; LDL: Low-density lipoproteins; VLDL: Very low-density lipoproteins; HDL: High-density lipoproteins; DPPH: 2,2-Diphenyl 1,1-picrylhydrazyl

Table 5 Scavenging of hydrogen peroxide radicals of MELCL

Sr. no	Concentration (µg/mL)	%Scavenging (Test)	%Scavenging (Standard)
1	50	23.07 ± 0.002	24.47 ± 0.003
2	100	25.87 ± 0.002	30.07 ± 0.003
3	150	31.46 ± 0.004	37.76 ± 0.003
4	200	39.16 ± 0.003	45.45 ± 0.001
5	250	47.55 ± 0.001	51.74 ± 0.001

Values are expressed as mean ± S.E.M; *n*=6
MELCL Methanolic extract of *Luffa cylindrica* (L.) leaves

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Experimental animals
Rats used during the procedure were from the animal house of the Nanded Pharmacy College, Nanded (CPCSEA Registration no: 1613/PO/a/12CPCSEA).

Plant authentication
The leaves of *Luffa cylindrica* Linn. (Cucurbitaceae) were collected in December from Ambajogai, Maharashtra, India. The plant was authenticated from the P.G. Department of Botany, N.E.S. Science College, Nanded, as *Luffa cylindrica* (L.) (Cucurbitaceae) with a voucher specimen no: S-1/21/02/15.

Authors' contributions
PR designed and performed the experimental part. SD guided for the work. The result analysis was performed by SS and PP. SP, DK, and AP contributed to the preparation of the manuscript. The authors have read and approved the final manuscript.

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Availability of data and materials
The data or analysis during the current study will be made available on request by the corresponding author.

Competing interest
The authors declare that they have no competing interests.

Ethics approval and consent to participate
All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) of Nanded Pharmacy College, Nanded Protocol Approval No. (3-VI/ 22/6/2014).

Consent for publication
Not applicable

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