ejpmr, 2021,8(5), 463-468

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANTI-HYPERLIPIDEMIC ACTIVITIES OF METHANOLIC EXTRACT OF MUSK MELON

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Article Received on 01/03/2021 **Article Revised on 20/03/2021** **Article Accepted on 10/04/2021**

ABSTRACT

Some of the plants, which we consume as part of our diet, are rich in bioactive compounds, which can heal various ailment, or it may supplement the action of the drugs, which we administer. Cucumis melo Linn is a fruit used in a different part of the world. It possesses various medicinal properties. The present study mainly focuses on the anti hyperlipidemic activity of the methanolic extract of muskmelon in high fat diet induced hyperlipidemia in Wistar albino rats. The result reveals a significant reduction in total cholesterol and Low-Density Lipoprotein (LDL). At the same time, an increase in High-Density Lipoprotein (HDL) levels compared to control and positive control group was observed. Combination of standard drug with test also produced a significant effect. The dose of the standard was reduced to half, and it was combined with a low dose of MECM. The results concluded that methanolic fruit extract (400 mg/kg) have potent anti-hyperlipidemic activity in high fat diet-induced hyperlipidemia model, when compared with Simvastatin, treated group.

KEYWORDS: Cucumis melo, Low Density Lipoprotein, High Density Lipoprotein, Cholesterol, Hypolipidemic.

1. INTRODUCTION

Mother Nature, has been showering blessings on humankind in an infinite variety of forms is a matter of cent per cent certainty. The knowledge about valuable plants was orally transferred and have not been documented.[1] Plant based natural drugs are an important source of therapeutic agents because of their availability, non-toxic nature and cheap cost than modern medicine.[2] Cucumis melo Linn is a fruit used in a different part of the world. The herb is with angular, scabrous stem, orbicular-reniform leaves which is simple soft hairy with tendrils. Its aerial parts, fruit pulp, seeds, seed oil, and roots are used medicinally for the treatment of various diseases.[3,4] It possesses various medicinal properties. Its nephroprotective, antimicrobial, anthelmintic, antioxidant, cytotoxic activity, anti-hyperlipidemic, analgesic, anti-inflammatory, diuretic, thyroid stimulatory activity has been established by research studies.[3] The fruit is having astringent properties along with cooling, demulcent action. It is also diuretic, aphrodisiac, emmenagogue and galactagogue. Various ailments of the excretory system have been treated with fruits for several centuries in conditions like ulcers in the urinary tract, dysuria, and suppression of urine. The methanolic extract of the seeds is particularly active due to the presence of phenolic compounds, particularly flavonoids.[5]

Hyperlipidemia is a condition whereupon there is an elevation in the concentration of cholesterol or triglycerides carrying lipoproteins in plasma. These lipid molecules get layered in the arterial wall thereby restricting the cardiovascular circulation.[6] Hyperlipidemia is an important factor for atherosclerotic cardio vascular diseases.[7] Hyperlipidemia is in connection with oxidative stress and inflammation. Studies suggest that atherosclerosis and hyperlipidemia is strongly associated.[8] Fatty material, atherosclerotic plaque and reactive oxygen species (ROS) play an important role in coronary artery disease. These species react with the biomolecules such as lipids, proteins and nucleic acids, resulting in change of their structure and functions.[9] Superoxides damage the membrane integrity and also inactivates nitric oxide, which is an endothelial vasoactive factor, all together causes endothelial dysfunction and finally tissue necrosis.[10]

Recently there is a drastic increase in studying the application of medicinal plants in the treatment of hyperlipidemia and cardiovascular diseases. From which, phenolic compounds were found to be promising

candidate for reducing hyperlipidemia. Reports suggest that flora with phenolic compounds, tannins and flavonoids are helpful in lowering hyperlipidemia.[11,12]

The present study aims to investigate the anti-hyperlipidemic activity of the methanolic extract of whole fruit. The study was performed in high fat diet induced hyperlipidemic rat models.

2. MATERIALS AND METHODS

2.1 Chemicals

The chemicals and drugs used for the study were of Pharmacopoeia/ analar or HPLC grade as required by the nature of experiment or extraction or as the case may be. They were purchased from licensed distributors of Central Drug House, Himedia Labs or Sigma-Aldrich chemicals.

2.2 Experimental Animals

The animals used for the study maintained under the standard animal house conditions like housing facilities, bedding materials, temperature, light and humidity conditions, noise-free atmosphere, feed, drinking water facilities, sanitation, etc. The animals were handled only in ethical and humane ways. No animals were subjected to unnecessary confinement or suffering or pain unless otherwise, the experimental protocol warrants it. Adherence to guidelines stipulated by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India were followed in the present study. The experimental protocols followed were approved by the Institutional Animal Ethics Committee of Centre for Professional and Advanced Study (CPAS), Department of Pharmaceutical Sciences Cheruvandoor-Ettumanoor as per order No. IAEC/PHD/DPS/2018-01

2.3 Procurement of research raw material

The material used for the study was fruits of *Cucumis melo* Linn (Family: Cucurbitaceae) were purchased from Vadanerkunam, Tindivanam T.K, Villupuram Dt, Tamilnadu and identified and authenticated by experts of Department of Botany, St. Berchmans College, Changanacherry, Kottayam-686101.

2.4 Preparation of extract

The fruits of *C. melo* were cut into pieces with the stainless steel knife. Weighed approximately 100 g, (including the seeds) and the fruit pieces together with the seeds were dried in an oven at 60^0C. The dried fruits were then comminuted to a coarse powder, which was then extracted with absolute methanol by hot continuous percolation process. The process of extraction was repeated with fresh powder, and the entire alcoholic extractives were combined, and most of the solvent was recovered by distillation under reduced pressure. The combined extracts were then evaporated under vacuum to form a soft extract. The final extract was weighed, re-heated and again weighed to make sure that it was free from alcohol. The yield was calculated as percentage weight per weight of powder used.

2.5 Anti-hyperlipidemic study

2.5.1 Composition of the high-fat diet

The high-fat diet was prepared by mixing coconut oil and Indian vanaspati ghee in the ratio of 1:3. It was given to the rat at a dose of 400 mg/Kg/day using oral gavage tube.[13]

2.5.2 Preparation of doses

The powder of the standard formulation as well as test (passed through sieve no.120) were suspended in a carboxymethyl cellulose (CMC, 0.5%). It was administered orally by gavage in volume not greater than 1 ml/100 g body weight.

2.5.3 Experimental protocol

36 Albino Wistar rats were used to conduct the study. The animals were divided into six groups, and each group contained six animals. The rats in different groups except control group were fed with high-fat diet once a day in a quantity of 400 mg/kg body weight/day. It was given using an intragastric tube for 30 days. A uniform suspension of fruit extract was prepared every day using 0.5% w/v sodium carboxymethyl cellulose.

The control group was given food and water ad libitum during the study period 2 ml of 0.5% w/v sodium carboxymethyl cellulose was also provided. The positive control group was fed with a high-fat diet along with a normal diet for 30 days. The high dose extract-treated group received the high-fat diet with regular food for thirty days. Fruit extract 400 mg/kg body weight /day was also given from 16th day onwards. Similarly, the low dose extract-treated group received fruit extract 200 mg/kg body weight /day from the 16th day onwards. Standard treated group[14] received Simvastatin in the dose of 10 mg/kg from the 16th day onwards. It was also freshly prepared using 0.5% w/v sodium carboxymethyl Cellulose. Animals on combination therapy received 200 mg/kg of MECM with Simvastatin in the dose of 5 mg/kg from the 16th day. On the first day and at the end of the study, the blood was collected by retro-orbital puncture and was subjected to biochemical analysis. The study design for hyperlipidemic activity is given in Table 1.

2.5.4 Estimation of serum cholesterol levels

On the first day and 30th day, the blood was collected by retro-orbital puncture and serum was separated. Levels of total cholesterol, High-Density Lipoprotein Cholesterol (HDL), Low-Density Lipoprotein Cholesterol (LDL) were measured.

Estimation of total cholesterol, high-density lipoprotein and low-density lipoprotein was carried out as the described methods.

2.5.4.1 Estimation of total cholesterol[15,16]

Cholesterol reacts with a hot solution of cholesterol reagent (ferric perchlorate, ethyl acetate and sulphuric acid) and gives lavender coloured complex which is measured at 560 nm. Reagents were mixed well, and the test tubes were kept immediately in the boiling water bath exactly for 90 sec. After that, test tubes were cooled quickly to room temperature under running tap water. Optical density (OD) of the standard and test were measured against blank on a colourimeter with a yellow-green filter at 560 nm.

$$Total\ serum\ cholesterol = \frac{Absorbance\ of\ test\ X\ 200}{Absorbance\ of\ standard}$$

2.5.4.2 Estimation of HDL-cholesterol[15,16]

On the addition of the precipitating reagent to the serum, followed by centrifugation, HDL fraction remains in the supernatant while the lipoprotein precipitates out.

$$HDL\ cholesterol\ \left(\frac{mg}{dl}\right) = \frac{Absorbance\ of\ test\ X\ 50}{Absorbance\ of\ standard}$$

2.5.4.3 Estimation of LDL-cholesterol

$$LDL\ (mg/dl) = Total\ Cholesterol - (HDL + VLDL)$$

3. RESULTS AND DISCUSSION

Oral administration of MECM (200 mg/kg, 400 mg/kg) and combination with standard drug significantly reduced Total cholesterol and LDL and there was an increase in HDL for standard treated and test groups. Results are furnished in table 2 and 3.

3.1 Total Cholesterol

The total cholesterol level in the control group was found to be increased by 0.35 ± 0.1562. This change was not statistically significant. It was found that total cholesterol levels in all other groups, which were fed with the high-fat diet, increased significantly ($p< 0.001$) on day 30 when compared with the increase of total cholesterol level in control group rats. The highest increase in the value of cholesterol was observed in the positive control group, 13.2 ± 1.727. The total cholesterol of animals in the standard group, which was treated with Simvastatin, showed an increase of 3.090 ± 0.2160. But when compared with positive control group there was a decrease. It was found to be statistically significant ($p<0.001$) in comparison with the positive control. Treatment with MECM at a low (200 mg/kg) and high (400 mg/kg) dose levels were found to produce an increase in total cholesterol in animals fed with the high-fat diet. The cholesterol levels in these groups were found to be increased by 5.162 ± 0.2265 and 9.853 ± 0.3667, respectively. But when compared with the positive control group animals, there was a reduction in the total cholesterol levels and it was found to be significant at $p<0.05$ and $p<0.001$, respectively. Group VI animals, which were fed with the high-fat diet, and treated with a combination of the low dose of MECM and Simvastatin showed an increase in cholesterol levels, which was found to be 6.927± 1. 159.At the same time in comparison with the corresponding value of the positive control group, there was a reduction which was found to be significant at $p<0.01$.

3.2 Change in HDL and LDL cholesterol

HDL cholesterol level of the control group showed a mild increase of 0.033 ± 0.3190 at the same time, HDL cholesterol of positive control decreased by 2.917 ± 0.410. Simvastatin (10 mg/kg) treated group showed an increase of HDL by 1.80 ± 0. 4107.MECM 200 mg/kg body weight treated group showed an increase of 0.4833 ± 1.613, whereas, for MECM 400mg/kg body weight treated group, the increase was 1.3333 ± 0.3676. On combining Simvastatin 5 mg/kg with MECM 200 mg/kg produced an increase in HDL by 1.867 ± 0.4185.

The LDL cholesterol level also showed a mild increase of 0.30 ± 0.7878. The positive control showed a rise of 3.1 ± 0.282. The change in LDL was 0.2267 ± 0.3211 for the standard, which shows a decrease compared to the positive control. For MECM 200mg/kg body weight and 400mg/kg bodyweight, the value obtained was 0.4167 ± 0.5218 and 1.183 ± 0.4135, which was also less compared to the positive control.

Table 1:- Study design for hypolipidemic activity.

No	Group	Drugs Given From0-15 Days	Drugs Given From16-30 Days	Route
I.	Control	0.5% w/v of CMC (2ml)	0.5% w/v of CMC (2ml)	P.O
II	Positive control	HFD (400 mg/kg/day)	HFD (400 mg/kg/day)	P.O
III	High dose	HFD (400 mg/kg/day)	HFD (400 mg/kg/day)+ MECM 400 mg/kg/day	P.O
IV	Low dose	HFD (400 mg/kg/day)	HFD (400mg/kg/day)+ MECM 200 mg/kg/day	P.O
V	Standard	HFD (400 mg/kg/day)	HFD (400mg/kg/day)+ Simvastatin10 mg/kg/day	P.O
VI	Combination	HFD (400 mg/kg/day)	HFD (400mg/kg/day)+ Simvastatin 5 mg/kg/day+ MECM 200mg/kg/day	P.O

Table 2: Effect of MECM in total cholesterol of rats on the high-fat diet.

Sl no	Group	Increase in total cholesterol on day 30 in mg/dl (Mean ± SEM)	Statistics
1	Normal control (A)	0.35 ± 0.1562	
2	Positive control (B)	13.2 ± 1.727	A & B ***
3	MECM 200 mg (C)	5.162 ± 0.2265	A & C*** C & B*
4	MECM 400 mg (D)	9.853 ± 0.3667	A & D*** B & D*** C & D ^{ns}
5	Simvastatin 10 mg (E)	3.090 ± 0.2160	A & E*** B & E*** C & E*** D & E***
6	Simvastatin 5 mg + MECM 200 mg (F)	6.927± 1.159	A & F*** B & F*** E & F^{ns} C & F***
	*** Significant, p<0.001, * significant, p<0.05, ns not significant. ANOVA and post hoc test by Newmann Keuls multiple comparisons. F= 173.		

Table 3: Change in HDL cholesterol and LDL Cholesterol.

Sl no	Group	HDL Cholesterol level on day 30 (Mean ± SEM)	LDL Cholesterol level on day 30 (Mean ± SEM)
1	Control	0.033 ± 0.3190	0.30 ± 0.7878
2	Positive control	- 2. 917 ± 0.4102**	3.1 ± 0.2828**
3	Standard	1.80 ± 0.4107	0.2267 ± 0.3211**
4	MECM 200 mg/kg	0.4833 ± 1.163	0.4167 ± 0.5218
5	MECM 400 mg/kg	1.3333 ± 0.3676	1.183 ± 0.4135
6	STD + MECM 200 mg/kg	1.867 ± 0.4185	0.60 ± 0.2633
	** significant, p<0.05. ANOVA and Dunnets multiple comparisons with control. F = 9.24		

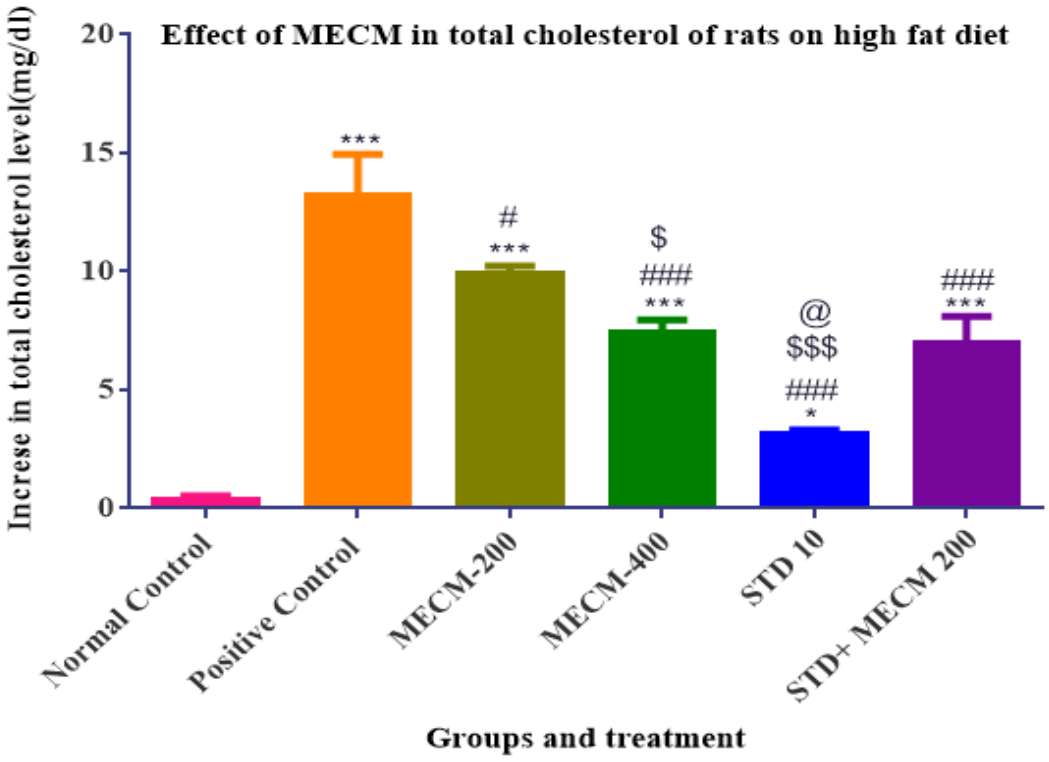

Fig: 1-Effect of MECM in total cholesterol of rats on the high-fat diet.

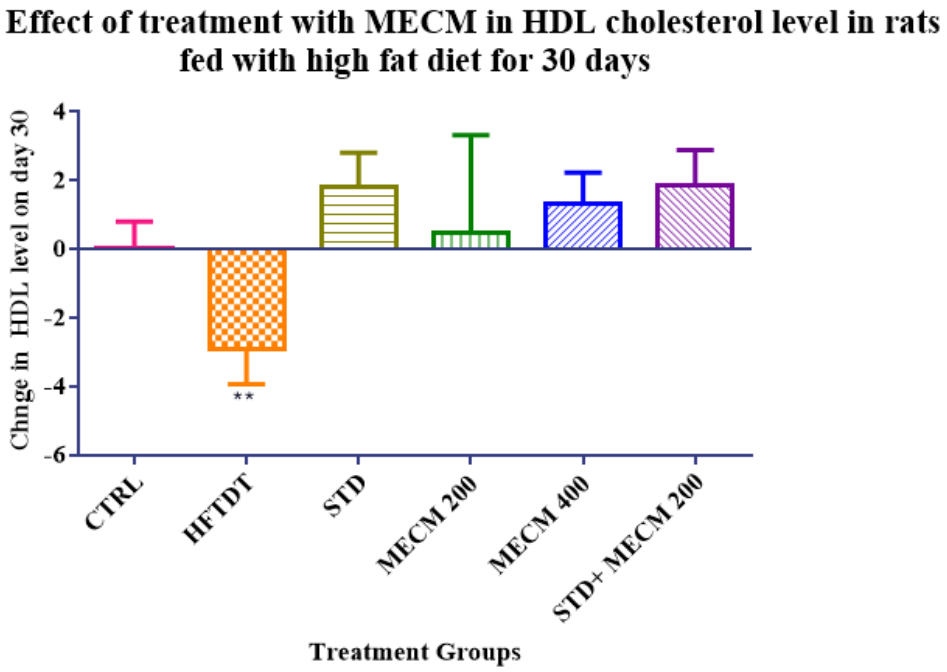

Fig: 2 Effect of MECM in HDL cholesterol levels in rat fed with high fat diet.

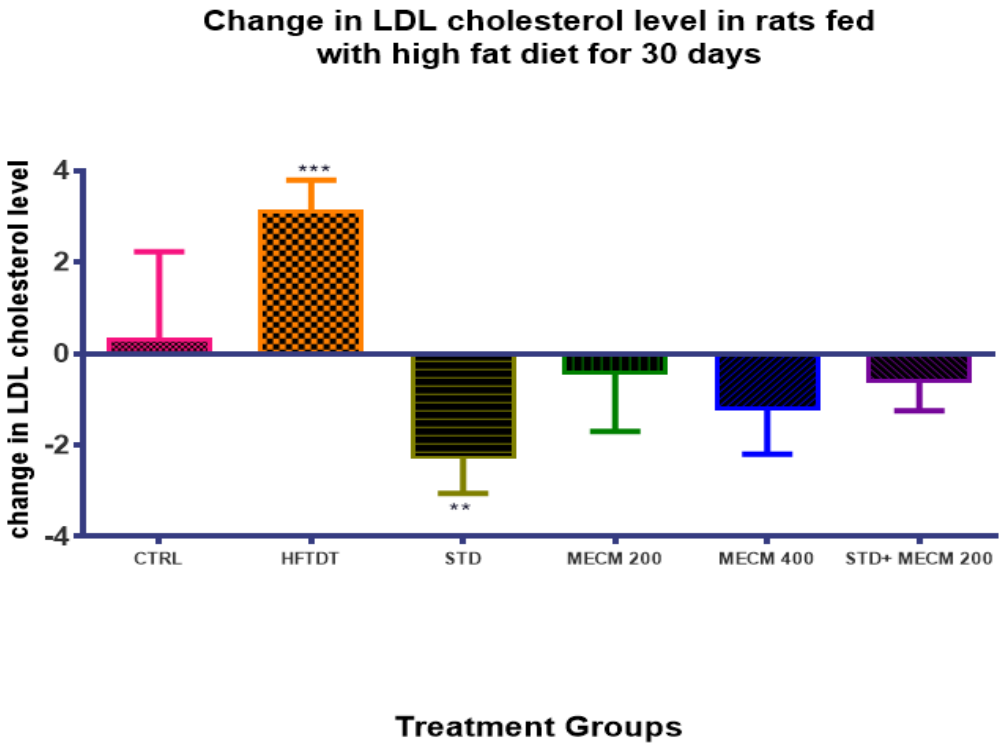

Fig: 3 Effect of MECM in LDL Cholesterol level in rats fed with the high-fat diet.

4. CONCLUSION

The results concluded that methanolic fruit extract (400 mg/kg) have potent anti-hyperlipidemic activity in high fat diet-induced hyperlipidemia model and which is equipotent activity when compared with Simvastatin treated group. There is a significant reduction in the total cholesterol and Low-Density Lipoprotein (LDL). At the same time, an increase in High-Density Lipoprotein (HDL) levels compared to control and positive control group was observed. Combination of standard drug with test also produced a significant effect. The dose of the standard was reduced to half, and it was combined with a low dose of MECM. The additive effect of the test drug may be due to its action by some other mechanism which requires further studies to prove.

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