IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 15, Issue 6 Ser. II (Nov. –Dec. 2020), PP 38-46
www.Iosrjournals.Org

A Study of Antihyperlipidemicactivity of *Averrhoea Carambola* L. (Starfruit) In Experimental Animal Receiving High Fat Diet.

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Abstract:
Background*: Hyperlipidemia is one of the leading causes of death in developed as well as developing countries. It is mainly due to the sedentary life style & eating habits of the people. In present study we have seen the effect of Averrhoea carambola L which is commonly known as star fruit on rats. Our study has been done to seen the effect of ethanolic extract of leaves of Averrhoea carambola L. on serum lipid in RABBITS which are receiving high fat diet*
Results*: It was observed that regular administration of high fat diet for 12 weeks to rabbits of Group B(Experimental Control) showed significant (P<0.0001) increase in total cholesterol (108.70±1.74), triglycerides (174.53±5.10) and LDL cholesterol (62.21 ± 3.27) while showing a significant decrease (P<0.0001) in the level of HDL cholesterol (11.66±0.96) in comparison to the Group A (Normal Control).*
In the test drug group concurrent administration of 200 mg/kg body weight/day of EELAC with high fat diet showed a significant (p<0.0001) decrease in total cholesterol (81.99 ±1.2), triglycerides (131.48±1.39), LDL (35.89±2.12) with a significant rise in HDL (19.34 ±0.95) as compared to the experimental control.
In second test drug group concurrent administration of 400 mg/kg body weight/day p.o.of EELAC with high fat diet showed a significant (p<0.0001) decrease in total cholesterol (73.42 ± 1.07), triglycerides (117.2±1.916), LDL (24.19 ± 1.99) with a significant rise in HDL (26.40± 1.57) as compared to the experimental control.
Discussion*: It has been seen that higher the doses of ethanolic extract, better the total cholesterol control in rabbits.*
Keyword*: hyperlipidemia, Averrhoea Carambola L, ethanolic extract*

Date of Submission: 16-11-2020 Date of Acceptance: 02-12-2020

I. Introduction

Hyperlipidemia is a major risk factor of atherosclerosis. The metabolic disorders that involve elevation in any lipoprotein species are termed as hyperlipoproteinemias or hyperlipidaemias. Acute pancreatitis and atherosclerosis are two major clinical sequelae of hyperlipidaemias. Hence, control of triglycerides can prevent recurrent attacks of this life-threatening disease.[1]

Defect in lipid metabolism is a primary reason of hyperlipidemia. Defect in lipoprotein lipase activity or the absence of the surface Apoprotein C-II causes defect in lipid metabolism. Various genetic abnormalities and environmental factors are the other causes of hyperlipidemia.[2]

Averrhoea carambola **L**. (commonly known as star fruit) belongs to the ***Oxalidaceae*** family and bears a great significance in traditional medicine

In traditional medicine, the ripe fruits or its juice are used as laxative, anti-pyretic, appetite stimulant. In the treatment of chicken pox, ringworm and headache the crushed leaves or shoots are used externally. Leaves are also useful in treating oliguria, boils, pyodermas, postpartum oedema, gastroenteritis and traumatic injury. Roots are used to treat arthralgia, chronic headache, epistaxis and spermatorrhea. Powdered seeds are used in cases of asthma and colic. Boiled flowers are also used as anthelmintic, in fever and malaria.[3]

Modern researches have revealed that star fruit has number of health benefits; anti-inflammatory activity, antimicrobial and antifungal activity, antitumor activity,antiulcer activity, negative inotropic and chronotropic effect, hypotensive activity, hypoglycaemic activity, hypocholesterolaemia, hypolipidemic activity, analgesic activity and antioxidant activity.[3,4,5]

Statin is a competitive inhibitor of HMG CoA reductase enzyme which catalyses the rate limiting step in cholesterol biosynthesis. Atorvastatin was taken as a standard drug for the study.[6]

II. Aim & Objective:

To determine the effect of ethanolic extract of leaves of ***Averrhoea carambola*** **L**. on serum lipid in RABBITS receiving high fat diet.

III. Material & methods:

The present study, "A STUDY OF ANTIHYPERLIPIDEMIC ACTIVITY OF *AVERRHOEACARAMBOLA* L. (STAR FRUIT)IN EXPERIMENTAL ANIMAL RECEIVINGHIGH FAT DIET." was conducted in the department of Pharmacology, Assam Medical College and Hospital, Dibrugarh

A) **DRUGS USED IN THE STUDY**:

1) Ethanolic extract of leaves of *Averrhoea carambola* L.
2) Atorvastatin was obtained from Lupin LTD, kartholi , Jammu.
3) Vehicle: Distilled water.

B) **REAGENTS USED IN THE STUDY:**

1) The kits for estimation of HDL-cholesterol, Total cholesterol and Triglyceride were obtained from Crest Biosystems, Goa, India.

C) **DIET USED IN THE STUDY:**

1) Normal diet: Standard animal diet consisting of bengal gram,wheat, maize and carrot in sufficient quantity and water ad libitum.
2) High fat diet: Mixture of coconut oil (from Marico Industries Ltd, Mumbai) and vanaspati ghee (from Ruchi Industries,Mumbai) in a ratio of 2:3 (v/v) at a dose of 10 ml / kg body weight per day. (P.O.)[7] (Shyamala MP *et al*., 2003)

D) **COLLECTION OF MATERIAL**:

1. PLANT MATERIAL:

Leaves of *Averrhoea carambola* L.(starfruit) were collected from areas in and around Dibrugarh District.

2. EXPERIMENTAL ANIMALS:

Twenty-five number healthy New Zealand white rabbit (*Oryctolaguscuniculus*) of either sex weighing of 1.5–2.5 kg were taken from the CentralAnimal House, Assam Medical College(Reg.No. 634/02/a/CPCSEA, dated 19/05/2002).

The study was duly permitted by the Institutional animal ethics committee (IAEC), Assam Medical College, Dibrugarh vide approval no **IAEC/AMC/16 dated09/11/2017**. The study was conducted keeping in view with the CPCSEA(Committee for the Purpose of Control and Supervision on Experiments on Animals) guidelines.

E) METHOD OF PREPARATION OF ETHANOLIC EXTRACT OF LEAVES OF *AVERRHOEA CARAMBOL*A L. (EELAC):

The ethanolic extract of leaves of *Averrhoea carambola* L. was obtained by the method of percolation as described by SS Handa *et al*.(2008). The leaves were collected, washed and dried in shade on a drier table and grounded to fine powder in electric mixture and grinder. Sufficient amount of the powdered leaves was moistened with an appropriate amount of 90% ethanol and allowed to stand for approximately 4 h in a well closed container, after which the mass was packed and the top of the percolator was closed. Additional amount of 90% ethanol was added to form a shallow layer above the mass, and the mixture was allowed to macerate in theclosed percolator for 24 h. The outlet of the percolator then was opened and the liquid contained therein was allowed to drip slowly. Additional amount of 90% ethanol was added as required, until the percolate measures about three-quarters of the required volume of the finished product. The marc was then pressed and the expressed liquid was added to the percolate. Sufficient amount of 90% ethanol was added to produce the required volume, and the mixed liquid was clarified by standing followed by decanting.[8]

The extract was collected in glass petri dishes, further dried in a vacuum desiccator and finally stored in airtight glass containers in a refrigerator at 2-8 ºC for use in the experiments. A final yield was 150.6 grams of EELAC.

I) **METHOD OF PREPARATION OF HIGH FAT DIET**:

Edible coconut oil (from Marico Industries Ltd, Mumbai) and vanaspati ghee (from Ruchi Industries, Mumbai) were purchased from the market and a mixture of the two was prepared in a ratio of 2:3 v/v respectively as per method of Shyamala MP *et al.* (2003).[7]

J) **METHOD OF INDUCING HYPERLIPIDAEMIA IN RABBTS**:

Rabbits are susceptible to hypercholesterolemia after excessive cholesterol feeding.[9]A high fat diet, consisting of coconut oil and vanaspati ghee, in a ratio of 2:3 v/v at a dose of 10 ml/kg body weight, was fed to the

animals, per orally, daily, in addition to normal diet for a period of 12 weeks[70]for the induction of hyperlipidemia

K) Grouping and treatment schedule:

Group A: Normal control- Received normal diet.

Group B: Experimental control- Received high fat diet at a dose of 10mg/kg bodyweight per day mixed with normal diet.

Group C1: Test drug group- Received high fat diet mixed with normal diet plusethanolic extract of leaves of *Averrhoea carambola* L.(EELAC) at a dose of 200 mg/kg/day orally.

Group C2: Test drug group- Received high fat diet mixed with normal diet plusethanolic extract of leaves of *Averrhoea carambola* L.(EELAC) at a dose of 400 mg/kg/day orally.

Group D: Standard drug group- Received high fat diet mixed with normal dietplus Atorvastatin at a dose of 2.1mg/kg/day orally.[10]

All the animals used for the experiment were kept under observation for daily food intake. The drugs were administered to the animals in the doses given above orally, once daily, for 12 weeks by means of intragastric feeding tube in the volume of 5 ml/kg body weight.
At the end of 12 weeks, body weights of all animals were measured and they were kept fasting for 18 hours.

IV. Results:

The study was conducted in the department of pharmacology, Assam Medical College and Hospital, Dibrugarh. The results and observations of this study are discussed below. This study was carried out with an attempt to evaluate the effect of ethanolic extract of leaves of *Averrhoea carambola*L. (EELAC) on serum lipid profile in rabbits fed with high fat diet in comparison to the standard drug atorvastatin.

The results obtained from the study have been summarized in tables numbered 5.1 to 5.5 and the observations are plotted in bar/line diagrams in figures numbering as 5.1 to 5.4.

The values obtained from the study were expressed in specific unit, such as for lipid level in mg/100ml&body weight was expressed in grams as mentioned in the respective tables. Results of estimations were plotted as Mean SEM (Standard Error of Mean) of five animals at a time from each group.

The statistical significance between groups was analysed separately using One-Way Analysis of Variance (ANOVA), followed by Bonferroni's multiple comparison test and paired t-test wherever required. The significance was expressed by '*p*' values, as mentioned in the tables. '*p*' values of <0.05 were considered as significant.[10]

Table 1: PHYTOCHEMICAL SCREENING OF EELAC.

Constituent	Present /Absent
Alkaloids	Present
Flavonoids	Present
Tannins	Present
Saponins	Present
Diterpenes	Absent
Phytosterols	Present
Phenols	Present

ACUTE TOXICITY TEST OF EELAC:

No mortality was recorded among the rats at the maximum dose of 2000mg/kg (all 5 animals survived at 2000mg/kg). Hence, the LD50 can be said to be above 2000mg/kg. One tenth (200mg) and two-tenth (400 mg) of this dose tested were selected for the experiments arbitrarily.

(A)**EFFECT OF EELAC ON SERUM LIPID PROFILE IN RABBITS FEDWITH HIGH FAT DIET**:
Table 5.2 and fig 1(a – d) show mean serum levels (in mg/dl) of lipid parameters i.e. serum total cholesterol, serum triglyceride, serum HDL cholesterol, serum LDL cholesterol in different groups at the end of 12 weeks of drug administration.

Table 2: EFFECT OF EELAC ON SERUM LIPIDS AT THE END OF THE12TH WEEK OFEXPERIMENT
Values are expressed as MEAN SEM (n=5)
One Way ANOVA followed by Bonferroni's Multiple Comparison test is done.

Groups	TEST RESULT (mg/dl)			
	Serum total cholesterol (mg/dl)	Serum triglyceride (mg/dl)	Serum high density lipoprotein (mg/dl)	Serum low density lipoprotein (mg/dl)
Group A (Normal control)	43.69 ± 0.98	61.06 ± 0.92	25.75 ± 0.96	5.72 ± 1.70
Group B (Experimental control)	108.70 ±1.74[a]	174.53 ± 5.10[a]	11.66 ± 0.96[a]	62.21 ±3.27[a]
Group C_1 (Test drug 200 mg/kg)	81.99 ±1.2[ab]	131.48 ± 1.39[ab]	19.34 ± 0.95[ab]	35.89 ±2.12[ab]
Group C_2 (Test drug 400 mg/kg)	73.42 ±1.07[abc]	117.2 ± 1.916[abc]	26.40 ± 1.57[abc]	24.19 ±1.99[abc]
Group D (Standard drug)	66.94 ±1.07[abcd]	104.37 ±1.45[abcd]	33.34 ±1.10[abcd]	12.72 ±2.07[abcd]
F ANOVA df *p*	359.9 4, 20 <0.0001	245.6 4,20 <0.0001	51.37 4,20 <0.0001	93.94 4, 20 <0.0001

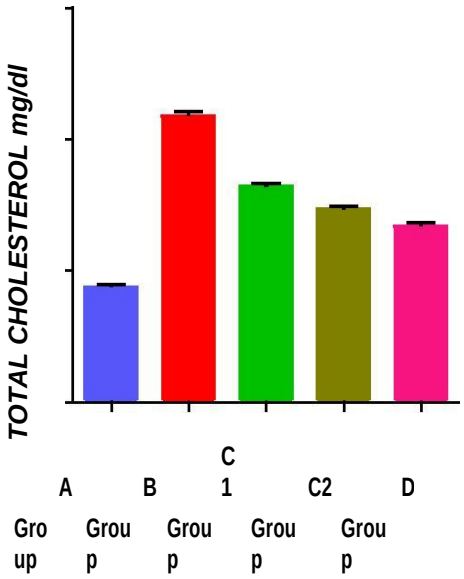
[a] p<0.05, when compared to the Group A (Normal control). [b]p<0.05, when compared to theGroup B (Experimental control).

[C]p<0.05, when compared to the Group C1 (Test drug 200 mg/kg) [d]p<0.05, when compared to the Group C2 (Test drug 400 mg/kg).

EFFECT OF EELAC on total cholesterol:

The average total serum cholesterol levels of rabbits belonging to the Group A (Normacontrol), Group B (Experimental control), Group C1(Test drug 200 mg/kg), Group C2 (Test drug 400 mg/kg) and Group D (Standard drug) were 43.69 ± 0.98, 108.70 ± 1.74, 81.98 ± 1.2, 73.42 ± 1.07, 66.94 ± 1.07 mg/dl respectively. The percentage of reduction of serum cholesterol in Group C1 (Test drug 200 mg/kg), Group C2 (Test drug 400 mg/kg) and Group D (Standard drug) were 24.58%, 32.46% and 38.41% respectively as compared to Group B (Experimental control).

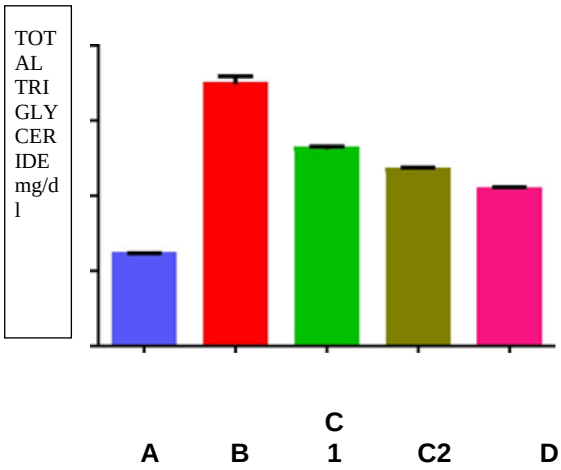
Fig: 1 (a)



EFFECT OF EELAC ON TRIGLYCERIDES:

The serum triglyceride levels of rabbits belonging to the Group A (Normal control), Group B (Experimental control) , Group C1 (Test drug 200 mg/kg), Group C2 (Test drug 400 mg/kg) and Group D (Standard drug) were 61.06 ± 0.92mg/dl, 174.53 ± 5.10mg/dl,131.48 ± 1.39mg/dl,117.2 ± 1.916mg/dl, 104.37 ± 1.45mg/dl respectively. The percentage of reduction of serum triglyceride in Group C1 (Test drug 200 mg/kg), Group C2 (Test drug 400 mg/kg) and Group D (Standard drug) were 24.66%, 32.96% and 40.19% respectively as compared to Group B (Experimental control)

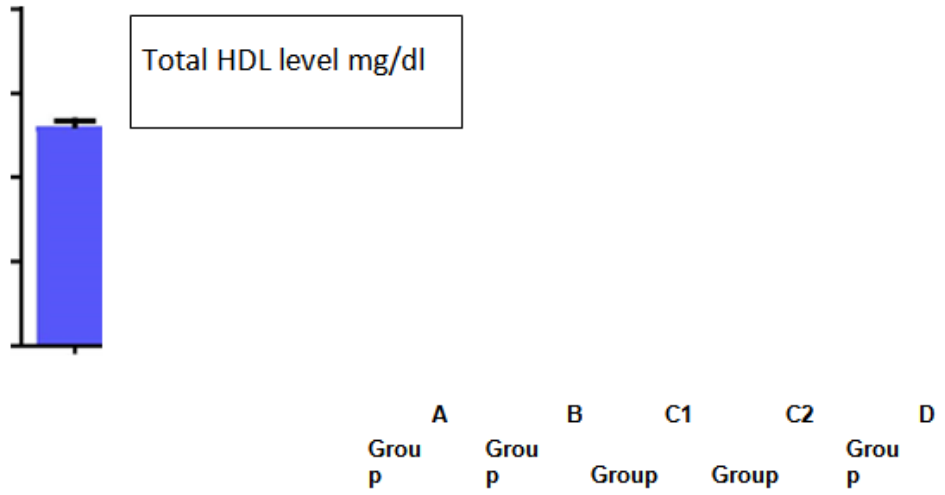
Fig: 1 (b)



EFFECT OF EELAC ON HDL LEVELS:

The serum high density lipoprotein (HDL) cholesterol levels of rabbits belonging to the Group A (Normal control), Group B (Experimental control), Group C1 (Test drug 200 mg/kg), Group C2 (Test drug 400 mg/kg) and Group D (Standard drug) were 25.75 ± 0.96mg/dl, 11.66 ± 0.96mg/dl, 19.34 ± 0.95mg/dl, 26.40 ± 1.57mg/dl and 33.34 ± 1.10 mg/dl respectively. The percentage of increase of serum high density lipoprotein (HDL) cholesterol in Group C1 (Test drug 200 mg/kg), Group C2 (Test drug 400 mg/kg) and Group D (Standard drug) were 66.2%, 126.328% and 185.75% respectively as compared to Group B (Experimental control).

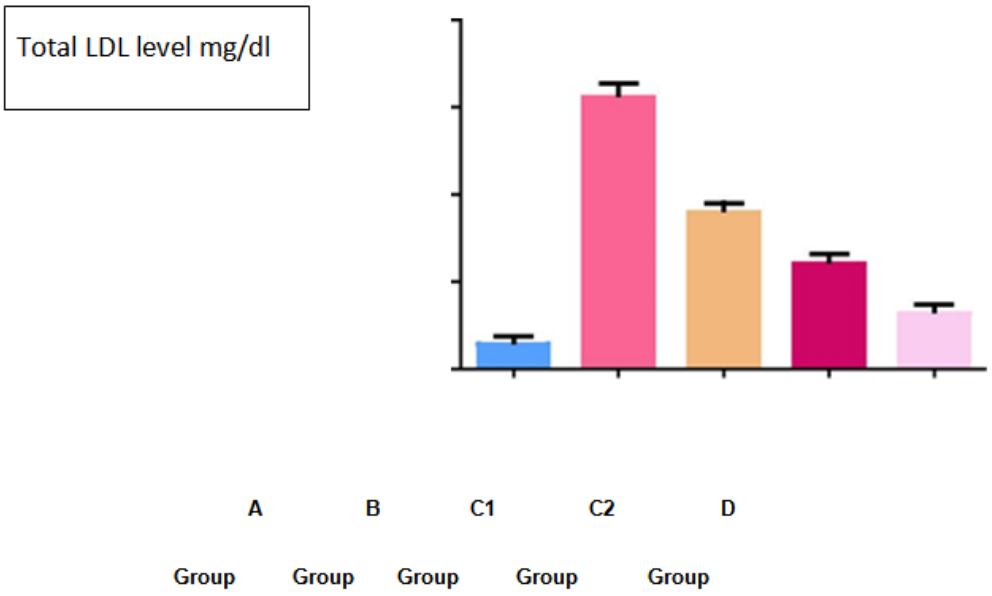
Fig: 1 (c)



EFFECT OF EELAC ON LDL LEVELS:

The serum low density lipoprotein (LDL)cholesterol levels of rabbits belonging to the Group A (Normal control), Group B (Experimental control), Group C1 (Test drug 200 mg/kg), Group C2 (Test drug 400 mg/kg) and Group D (Standard drug) were 5.72 ± 1.70mg/dl, 62.21 ± 3.27mg/dl, 35.89 ± 2.12mg/dl, 24.19 ± 1.99mg/dl, 12.72 ± 2.07mg/dl, respectively. The percentage of reduction of serum low density lipoprotein (LDL) cholesterol in Group C1 (Test drug 200 mg/kg), Group C2 (Test drug 400 mg/kg) and Group D (Standard drug) were 42.3%, 61.11% and 79.55% respectively as compared to Group B(Experimental control).

Fig: 1 (d)



V. Discussion:

Hyperlipidemia is a major cause of coronary artery disease and atherosclerosis. Oxidative stress also plays an important role in the atherogenic process. The aim of the present study was to evaluate antihyperlipedemic activity of ethanolic extract of leaves of ***Averrhoea carambola*L.*(STARFRUIT)*(EELAC)** on fatty diet induced hyperlipidemic states in rabbits in comparison to a standard hypolipidemic agent Atorvastatin and normal rabbits that received normal diet alone. The results of above-mentioned groups were compared with Group B (experimental control) that received only high fat diet.

In this study, hyperlipidemia was induced in rabbits by feeding them a high fat diet, which was a mixture of coconut oil and vanaspati ghee in the ratio of 2:3 v/v at a dose of 10ml/kg body weight. [7](Shyamala

MP *et al.*,2003)

It was observed that regular administration of high fat diet for 12 weeks to rabbits of Group B(Experimental Control) showed significant (P<0.0001) increase in total cholesterol (108.70±1.74), triglycerides (174.53±5.10) and LDL cholesterol (62.21 ± 3.27) while showing a significant decrease (P<0.0001) in the level of HDL cholesterol (11.66±0.96) in comparison to the Group A (Normal Control).

A study done by Zhang Z*et al.* (2002), also reported the similar rise in lipid parameters in New Zealand white rabbits fed with a high fat diet (containing 1 g/100 g cholesterol). [11]

In group C1 (test drug group), concurrent administration of 200 mg/kg body weight/day of EELAC with high fat diet showed a significant (p<0.0001) decrease in total cholesterol (81.99 ±1.2), triglycerides (131.48±1.39), LDL (35.89±2.12) with a significant rise in HDL (19.34 ±0.95) as compared to the Group B (experimental control). The percentage of reduction of serum cholesterol caused by 200 mg/kg body weight/day p.o. of EELAC was 24.58%, while that of serum triglyceride and serum low density lipoprotein were 24.66% and 42.3% respectively. The percentage of rise in serum HDL – cholesterol caused by 200 mg/kg body weight/day p.o. of EELAC was 66.2%.

In case of Group **C2**after concurrent administration of 400 mg/kg body weight/day p.o.of EELAC with high fat diet showed a significant (p<0.0001) decrease in total cholesterol (73.42 ± 1.07), triglycerides (117.2±1.916), LDL (24.19 ± 1.99) with a significant rise in HDL (26.40± 1.57) as compared to the Group B (experimental control). The percentage of reduction of serum cholesterol caused by 400 mg/kg body weight/day p.o.of EELAC was 32.46%, while that of serum triglyceride and serum low density lipoprotein were 32.96% and 61.11% respectively .The percentage of rise in serum HDL – cholesterol caused by 400 mg/kg body weight/day p.o.of EELAC was 126.328%.

In the Group D (standard drug), administration of Atorvastatin (2.1mg/kg/day,orally)exhibited significant (P<0.0001) decrease in total cholesterol (66.94 ± 1.07), triglyceride (104.37 ±1.45) and LDL cholesterol (12.72 ± 2.07),while significant increase in HDL cholesterol (33.34 ± 1.1). From the results of the present study it was found that Group D (Atorvastatin2.1mg/kg/day, orally) has better efficacy against hyperlipidemia than the two test drug groups (Group **C1** and **C2**).

Among the test drug group's EELAC at the dose of 400 mg/kg body weight/day is more effective than EELAC at the dose of 200 mg/kg body weight/day.

Ma L*et al.* (2015) have demonstrated the lipid lowering effect of flavonoids isolated from the leaves of *Zanthoxylum bungeanum* (ZLE) on male homozygous apoE-/- mice. They hypothesized that the strong DPPH (2,2-diphenyl-1-picryl-hydrazyl) radicals scavenging activity of (ZLE) was due to the high amount of flavonoids present in the plant. Flavonoids like; hyperoside, rutin and isoquercitrin are reported to have antioxidant property due to which they are able to inhibit the process of atherosclerosis and cardiovascular diseases. In several studies they have showed protective activity against lipid peroxidation and hyperlipidemia which may be due to their free radical scavenging activity. [12]

Flavonoids have been reported to reduce HMG-CoA reductase activity, inhibit cellular cholesterol esterification, synthesis of triacylglycerol, phospholipids and the secretion of apo B from the liver. All these actions result hypocholesterolemic activity of flavonoids. [13]

Citrus flavonoids play an important role in lowering serum LDL level by increasing hepatic uptake and degradation of LDL. [14]

Citrus flavonoids, naringenin and hesperitin, have also reported to reduce apo B secretion in hepatocytes. The reduction of hepatic apo B production by polyphenols may be exerted through their binding with the plasma membrane transport P-glycoprotein, which inhibits cholesterol esterification, decreasing the incorporation of CE (cholesteryl ester) into nascent VLDL. [15]

Moresco HH *et al.* (2012), has reported that ***Averrhoea carambola*L.** leaves contain high amount of phenolic compounds, mainly flavonoids. Results of a study showed that leaves of *Averrhoea carambola*L. contains; β-sitosterol and apigenin-6-*C*-β-L-fucopyranoside, two *C*- glycosyl flavones, and apigenin-6-*C*-(2"-*O*-α-Lrhamnopyranosyl)-β-L-fucopyranoside. [16]

Therefore, in our study the flavonoids present in leaves of ***Averrhoeacarambola*L.**(STARFRUIT) may act in a similar manner and reduce serumcholesterol and LDL levels.

Ya-Mei Y*et al.* (2002) have demonstrated hypocholesterolemic action of β-sitosterol a constituent of seed kernel of *Caesalpinia bonducella*. β-sitosterol is assumed to inhibits the intestinal absorption of cholesterol and increase the catabolism of cholesterol to bile acid. [17]

As leaves of ***Averrhoea carambola*L.** contain β-sitosterol, the plant may exert the antihyperlipedemic activity by a similar mechanism.

Various animal studies have suggested that polyphenols may exert protective effect on the cardiovascular system via decreasing the absorption of cholesterol by interacting with cholesterol carriers and transporters present across the brush border membrane. It is also reported that majority of polyphenol reduces the production of hepatic lipoproteins. [15]

From the scientific evidences it is concluded that, dietary polyphenols mainly flavonoids though their anti-inflammatory and anti-oxidative properties can prevent the process of atherosclerosis. Ciccone MM *et al.* (2015), have demonstrated the hypolipidemic and hypocholesterolemic effect of Bergamot polyphenols. They suggested that, polyphenols act by preventing the oxidation of LDL and formations of foam cells and polyphenols induces the hydrolysis of the peroxidised lipids from the atherosclerotic plaque. [18]

Akazome N Y*et al.*(2005) have reported that apple polyphenols have the lipid lowering property in healthy subjects and polyphenols can significantly reduce the LDL cholesterol and increases the HDL cholesterol level. They suggested the possible mechanism of lipid lowering effect of apple polyphenols was due to its binding ability to the cholesterol and/ or bile acid, which leads to increased excretion through faeces. They also suggested that polyphenols also inhibit the enterohepatic circulation of bile acid and cholesterol. It has also reported that apple polyphenols inhibit the of cholesterol micelle formation, thereby inhibit lipid uptake in the intestine. [19]

According to various studies, it is well known that phytochemicals like; phenolic compounds and phytosterols can prevent various cardiovascular diseases by improving the functions of endothelium and by reducing prothrombic and inflammatory status. From the epidemiological studies it was noticed that the populations who take the diet rich in phytochemicals reported less incidence of cardiovascular diseases. It was hypothesized that these effects are due to anti-oxidative, anti-inflammatory, antiprothrombotic and lipid lowering properties of phytochemicals. Various studies done on animals have shown that cholesterol reduction by phytosterols is due to up-regulating hepatic ABCG5 transporters.[20]

As the leaves of ***Averrhoea carambola*L.**containpolyphenols, the antihyperlipidemic activity of the plant is also contributed by the presence of polyphenols and phytosterols.Marrelli M *et al.* (2016) reported that Trillin, a steroidal saponin isolated from*Dioscoreanipponica* Makino rhizome, have anti-hyperlipidemic activity. They foundthat the levels of LDL, cholesterol and HDL came back to the normal conditions after administration of saponin rich extract. [21]

As saponin is a constituent of leaves of ***Averrhoea carambola*L**., the antihyperlipidemic activity also may be due to the presence of saponins.

Although no study has been undertaken to fully evaluate the molecular and biochemical basis of antihyperlipidemic action of***Averrhoea carambola*L**. (STARFRUIT), it can be presumed that the presence of bioactive phytochemicals and antioxidants in leaves of *Averrhoea carambola*L. (STARFRUIT), is responsible for its antihyperlipidemic activity.

VI. Conclusion:

The antihyperlipidemicaction of*AVERRHOEA CARAMBOLA* L. may be due to the bioactive phytochemical presentin it, which exert a positive effect on serum lipids by controlling different steps of lipid absorption, its transport, metabolism and free radical scavenging effect.

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Dr Arundhati Lahon, et. al. "A Study of Antihyperlipidemicactivity of Averrhoea Carambola L.(Starfruit) In Experimental Animal Receiving High Fat Diet.*" IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 15(6), (2020): pp. 38-46.