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Hypolipidemic Effect of *Aegle Marmelos* on Hyperlipidemic Rat Models

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Abstract: The growing challenges of modernization have resulted in human being readjusting their customary behavior by modifying their dietary and lifestyle preferences which has adversely impacted the lifestyle of man Due to impaired metabolism the human population has been engulfed by many ailments that have deleterious result on their health. Of these, one specific risk factor is hyperlipidemia that causes greater morbidity and mortality, in both young and elderly people and the major risk factor of developing cardiovascular disease.

It involves an imbalance of cholesterol levels including low density lipoprotein and high density lipoprotein in the blood .Management of dyslipidemia forms an important part for procedure for forestalling cardiovascular ailments, Currently available hypolipidemic drugs like statins, fibrates, niacin have been associated with number of side effects .Medicinal plants have always been considered a healthy source of life for all people due to its rich therapeutic properties .Indian medicinal plants are considered a vast resource of several pharmacologically active principles and active constituents, which are commonly used in home remedies against multiple ailments.

The present study was designed to investigate the hypolipidemic effect of Aegle marmelos plant extracts in high cholesterol diet induced hyperlipidemic rats. Ethanolic extract of Aegle marmelos were administered orally at dose of at a dose of 125 and 250mg/kg/day which shows significant lowering of serum levels cholesterol and triglycerides when compared to that of hyperlipidemic rats. The findings of the study reveals that ethanolic extracts of can effectively control the blood cholesterol and triglycerides levels in dyslipidaemic conditions. The main reason of this study was to find an alternative and safe drug for the management of hyperlipidemia.

Keywords: Medicinal plants, Aegle marmelos, Dyslipidemia, Cholesterol.

I. INTRODUCTION

Hyperlipidemia is a systemic disease that impairs the body in a generally unnoticeable, steady, progressive and systemic way .LDL and HDL regulate the amount of cholesterol in the body and an imbalance can increase the risk of cardiovascular events including myocardial infarction and stroke. Decreased concentrations of high density lipoprotein (HDL) cholesterol as well as qualitative changes in low density lipoprotein (LDL), together with raised triglycerides, are highlights of the metabolic disorders, increasingly recognized as an indicator of coronary heart disease.

The direct damage of hyperlipidemia can accelerate systemic arteriosclerosis, and it is an important risk factor for many diseases, such as stroke, coronary artery disease, myocardial infarction and cardiac sudden death [1]. The incidence of hyperlipidemia, a disorder of lipid metabolism, is presently increasing at a dramatic rate throughout the globe. According to a report by Global Burden of Diseases, it was estimated that 55 million deaths occurred in the world in 2017, of which approximately 17.7 million were due to cardiovascular disease. Number of death caused in India in 2017 is 2.63 million when compared to other diseases and it accounts for 14.6 9% percentage of the total Global share.

Management of dyslipidaemia forms an important part of procedures for forestalling cardiovascular ailments. Hypolipidemic drugs like Statins; Bile Acid Sequestrants (BASs), Niacin have been associated with number of side effects. They are linked with many adverse consequences, which comprise gastrointestinal events, musculoskeletal pain (including bilateral muscle pain, weakness, and inflammation), respiratory infections and headaches [2], [3]. Apart from the adverse effects of the antihyperlipidemic drugs, the high cost of these drugs is also a main drawback especially for the developing countries. These issues in treatment provide an opportunity to consider the use of natural products for patients. Attempts have been made during the last century to quantitatively measure the effect of each phytomedicine alone and in combinations and also determine the type of effect of the combination. Indian medicinal plants are considered a vast resource of several pharmacologically active principles and active constituents, which are commonly used in home remedies against multiple ailments [4].



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Medicinal plants of Indian origin are well thought-out as an immeasurable reserve of numerous pharmacologically active principles and phyto-constituents, which are commonly used in various remedies against several ailments[5]. Owing to the importance of plants in the medicine several efforts have been made to identify and validate the plant derived phytochemicals for the treatment of various diseases, and the consequence is that these days more than 25 percent of the modern medicines are directly or indirectly derived from plants[6].

The aim of the present work is to propose a preparation for the treatment of hyperlipidemea accelerated by high fat diet ,which is cost-effective, more effective than synthetic drugs and does not have any toxic or side effect .[7]*Aegle marmelos*, commonly known as Bael, (family: Rutaceae) is a plant of Indian origin having immense curative beneficial potential. The fruit is also used as a dietary supplements, it is also used to cure intermittent fever, mental disease, has hypoglycaemic, antifungal, anti microbial, analgesic, anti inflammatory, antipyretic, antidyslipidemic activity, anti proliferative activity, wound- healing activity, anti fertility, insecticidal activity etc[8],[9].It is known by the several other names in the different parts of the country and also outside of the country [10]. The *Aegle marmelos* plant is native to India and is grown in the Himalaya range to west Bengal, around foot hill of Uttar Pradesh, Bihar, Chhattisgarh, Madhya Pradesh, Uttaranchal, Jharkhand, the Deccan Plateau. *Aegle marmelos* is a slow-growing spiky tree, ~12 to 15 meters in height having petite trunk with thick, soft and scaly bark. [11]. The flowers are greenish white; sweet scented about 2.5 cm across. The fresh foliage is lustrous and pinkish-maroon in colour The fruits are round, pyriform oval or oblong, 5-20 cm in diameter, can have a thin, hard, woody shell or a more or less soft rind, grey green until the fruit is fully ripe, when it turns yellowish[12]. The scientific validations of numerous ethno medicinal uses of *A. marmelos*, which include antibacterial, antiviral, anti diarrheal, gastro protective, anti-ulcerative colitis, hepatoprotective, antidiabetic, cardio protective and radio protective effects and anticancer effects has been well documented.[13]

II. METHODOLOGY

The burden of hyperlipidemia in India is disturbingly high and is a cause of concern. Indians are not only at high risk of developing atherosclerotic cardiovascular disease; they usually get the disease at an early age and have poorer outcome in contrast to the western populations. Access to health care is also not approving in India, and the treatment remains expensive. Nowadays, human being face a myriad of multi-faceted heath related problem due to which we are getting addicted of synthetic medicine, which definitely gives the fast result but brings several new problem with them like side effect and adverse effect. On the other hand the herbal drugs obtained from plants like *Aegle marmelos* (Bael) (family: Rutaceae)) is much more valuable and safe comparatively those chemical.

A. Experimental animal

Male wistar rats weighing -200-280gm with 7.5 \pm 1.0 cm length are selected for experiments were used in present study. Five groups of six rats were housed in polypropylene cages under standard laboratory conditions. The rats were allowed to acclimatize for 15 days in an environmentally controlled room under standard environmental conditions (21 \pm 2°C, 55 \pm 5% humidity, 12 hr Light: Dark cycle). They were given standard rat pellets, tap water and high cholesterol diet according to their group.

B. Preparation of plant extract

The plant materials were prepared according to the Ayurvedic Pharmacopoeia of India (2001). Freshly harvested plant materials were washed, dried in the shade at room temperature and powdered. Each of the dried plant samples (2.6 kg each) was then soaked with absolute ethanol under reflux condition for the ethanol extract preparation. To this thick paste of both the samples, colloidal silicon dioxide was added and dried in vacuum tube dryer. The obtained plant extracts were stored in freezer at -20° C until further test. The quality control sample was prepared as 5 mg/ml of each extract in ethanol.

C. Induction of Hyperlipidemic Diet

The rats were fed on "In laboratory prepared enriched bread" having the composition given below and water *ad libitum* to ensure proper growth and reproduction as recommended by Templeton, Table I. The animal model for the current study was based on feeding of in laboratory prepared enriched bread + high fat diet, Table-II for 4 weeks. The serum cholesterol levels was elevated with high fat diet when compared to normal levels and the HDL-C levels were reduced significantly. [14]To confirm the induction of hyperlipidemia, blood samples were collected and lipid profile was determined using diagnostic kits.



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Sl. No.	Ingredients	Weight
1.	Wheat grains	1000 g
2.	Choker wheat 250 g	
3.	Grams grains	250 g
4.	Maize grains	250 g
5.	Soyabean grains	250 g
6.	Sundrop oil	50 g
7.	Milk powder	2 table spoon
8.	Jaggery(Gudd)	50 g

Table I: In laboratory prepared enriched bread

Table II: Composition of high fat diet

1.	Cholesterol	2%
2.	Sucrose	80 g
3.	Glucose	20 g
4.	Starch	50 g
5.	Coconut oil	5 ml/ 100 g
6.	Casein	30 g
7.	Cellulose	100 g
8.	Vitamin mix	20 g
9.	Sodium cholate	1%
10.	Propylthiouracil	0.2%
11.	Lard	15%
12.	Yolk powder	5%

D. Dose Preparation and Administration of Extracts.

The extracts of plants at dose of 125mg/kg b wt and 20mg/kg b wt were given to animals once in day along with HFD orally for4 weeks. One group continued to receive in laboratory prepared enriched bread as recommended by Templeton and constituted the NLC group (normolipidemic control rats); the other was fed with hyperlipidemic diets, in order to induce hyperlipidemia. All the rats had free access to food and water.

E. Animal groupings and experimental design

Table III shows that the hyperlipidemic rats were divided into five groups having six rats in each group:, one HLC group (hyperlipidemic control rats) and three HLT group (hyperlipidemic rats treated with two different doses of extract and atorvastatin (AVT) at a defined dose per kg of body weight). The rats in the atorvastatin treatment group were administered atorvastatin calcium tablets at a dose of 2.0 mg-Kg-1 -d -1 (Pfizer pharmaceuticals limited)

Group 1	NORMAL : Normolipidemic control rats
Group 2	HLC-Hyperlipidemic Rats
Group3	HLT ₁₂₅ : Hyperlipidemic rats treated with 125 mg of extract of <i>Aegle marmelos</i>
Group 4	HLT_{250} : Hyperlipidemic rats treated with 250 mg of extract <i>Aegle marmelos</i>
Group 5	HLT_{AVT} : Hyperlipidemic rats treated with atorvastatin at a dose of 2.0 mg/ kg of body wt

Table III: Animal Groupings and Experimental Design



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III. RESULT

The efficacy of medicinal plants in management of hyperlipidemia as an alternative drug is of great interest. These drugs from plant origin are priceless and safe as compared to those of synthetic chemicals due to their favorable effects on metabolic profile. The plant extract of two plants Aegle marmelos (Bael) (family: Rutaceae) have been well documented for their effectiveness in the alleviation of hyperlipidemia through its antioxidant and lipid lowering activities [15]. The changes in the blood glucose levels before and after receiving the treatment in normal, hyperlipidemic control and hyperlipidemic treated rats are listed in Table IV. The hyperlipidemic control (HLC) rats showed significantly higher level of glucose which is about 379.08 mg/dl more than normal control rats (NLC) Hyperlipidemic treated rats groups i.e., HLT_{AM-125}, HLT_{AM-250}, of the plants showed a significantly reduction in glucose levels, when compared to the normal control rats (NLC). The rats of A. marmelos treatment groups i.e., HLT_{AM-125} and HLT_{AM-250} showed a decrease of 176.67 mg/dl and 199.45 mg/dl in blood glucose levels respectively at the end of the experimental period.

radie rv. Effects of treatment on blood grucose levels in various rat groups						
	GROUPS	Blood glucose levels (mg/dl) in week				
		Pretreatment	Post-treatment			
		0	1	2	3	4
	NLC	132.75 ± 2.05	133.76 ± 2.36	131.52 ± 2.83	130.87 ± 2.35	132.64 ± 2.27
HLC		511.83 ± 3.86	513.49 ± 3.25	523.42 ±2.42	518.87 ± 2.43	519.87 ± 2.97
HLT _{AVT}		510.53 ± 2.86	460.82 ± 3.83	421.82 ± 2.84	343.33 ± 2.44	277.56 ± 3.55
A. ss	HLT _{AM-125}	511.12 ± 2.35	489.89 ± 3.88	455.84 ± 2.83	373.36 ± 2.47	334.45 ± 3.44
.marmelo :s	HLT _{AM-250}	509.88 ± 3.77	473.24 ± 2.95	436.84 ± 2.46	359.38 ± 2.23	310.43 ± 3.24

The effect of the two plant extracts on serum lipid levels on experimental rat groups have been presented in Table V. When compared with normolipidemic control rats (NLC), the hyperlipidemic control rats (HLC) had reasonably higher total cholesterol (TC) values by about 134.84 mg/dl and TGs values by about 357.02 mg/dl. The atorvastatin treated rats (HLT_{AVT}) showed a reduction of 113.92 mg/dl of serum TC level and 300.71 mg/dl of serum TGs level when compared to hyperlipidemic control (HLC) rats. These changes in biochemical parameters are as expected as, when the uncontrolled hyperlipidemic condition progresses, considerable alterations in total cholesterol and triglycerides values are predictable. Hyperlipidemic rats treated with lower dose of A. marmelos extract (HLT_{AM-125}) showed significantly lower values of serum TC by 43.01 mg/dl, when compared to the hyperlipidemic control (HLC) counterparts. Contrary to this, the higher dose of A. marmelos extract treatment (HLT_{AM-250}) showed superior lowering effects on serum TC by about 89.99 mg/dl also on serum TG by about 278.53 mg/dl when compared to the hyperlipidemic control (HLC) counterparts. Relative to normal control (NLC), the hyperlipidemic control rats (HLC) had higher value of low density lipoprotein (LDL) by about 26.17 mg/dl while diminished value of high density lipoprotein (HDL) by about 23.22 mg/dl.Hyperlipidemic rats treated with lower dose of A. marmelos extract (HLT_{AM-125}) showed significantly lower values of serum LDL by 12.00 mg/dl and higher value of HDL by 4.02 mg/dl when compared with the hyperlipidemic control (HLC) counterparts. The higher dose of A. marmelos extract treatment (HLT_{AM-250}) showed even better lowering effects on LDL by 21.89 mg/dl when compared to the hyperlipidemic control (HLC) counterparts as well as lower dose of A. marmelos extract (HLT_{WS-250}) treated rats and improved level of HDL by 15.84 mg/dl.

(GROUPS	TC (mg/dl)	TG (mg/dl)	HDL(mg/dl)	LDL(mg/dl)
NLC		76.03 ± 1.42	62.45 ± 0.75	66.05 ± 2.49	64.70 ± 2.28
	HLC	210.87 ± 1.65	419.47 ± 3.46	42.83 ± 2.85	90.87 ± 3.38
	HLT _{AVT}	96.95 ± 1.56	118.76 ± 3.54	60.29 ± 3.25	66.76 ± 3.77
A.ı elc	HLT _{AM-125}	167.86 ± 1.56	201.42 ± 3.38	46.85 ± 3.85	78.87 ± 3.89
marm >S	HLT _{AM-250}	120.88 ± 1.30	140.94 ± 3.65	58.67 ± 3.87	68.98 ± 3.98

Table VI: Effect of treatment on serum lipid levels in various rat groups

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IV CONCLUSION

Our present investigation demonstrated that the extracts from the two plants *Aegle marmelos* perform antihyperlipidemic effects compared to atorvastatin in a dose dependent manner and could promote a better health. Although there have been extensive research on this plants, there is still a lot of scope for further research, especially towards the molecular mechanism of antihyprtlipidemic activity. Mechanistic studies are expected to lead the way in the discovery of new agents with improved and intriguing pharmacological properties. The main finding of the study demonstrated that both the plant extracts perform effectiveness compared to atorvastatin in a dose dependent manner in prevention of hyperlipidemic condition and could promote a better health conditions. When compared the therapies for hyperlipidemia, the higher dose of both the plant extracts *i.e.*, *Aegle marmelos* have shown improvements lipid and glucose levels. Consequently, we can infer that application of *Aegle marmelos* plant extracts can be a supplement to the existing oral anti hyperlipidemic drugs. The data generated in this study adds information to the scientific knowledge and useful to the humanity and helped in understanding the presence of active phytochemicals in medicinal plants and their probable potential use in treatment of hyperlipidemic conditions.

Their active compounds may be further tested for their safety and efficacy to uncover their therapeutic potential in modem medicine. In conclusion, it is our deep confidence that the application of aforesaid plant having hypolipidemic, renoprotective, and hapatoprotective and antioxidant properties can be a supplement or an alternative to the existing oral antihyperlipidemic drugs.

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