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Original Article

Physiology Section

Protective effects of Ethanolic Extract of *Emblica Officinalis* (amla) on Cardiovascular Pathophysiology of Rats, Fed with High Fat Diet

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ABSTRACT

Introduction: Dietary high fat alters lipid profile and possibly induce sympatho-vagal imbalance. *Emblica Officinalis* is found to be potential antioxidant and possibly counteract hyperlipidemia induced lipid peroxidation.

Aim: To assess Ethanolic extract of *Emblica Officinalis* (EEO) as lipid lowering and cardiovascular protective agent against high dietary fat supplemented to experimental rats. Further to study a comparative analysis between EEO and atorvastatin on hyperlipidemia and cardiovascular integrity.

Material and methods: EEO was prepared and phytochemical analysis was done. Rats were divided into five groups, having six rats in each group as following; group I-control (20% fat); group II (+ EEO 100 mg/kg body wt); group III (fed with high fat diet; 30% fat); group IV (fed with high fat diet; 30% fat + EEO100mg/kg body wt) and group V (fed with high fat diet; 30% fat + atorvastatin 4mg/kg body wt). The treatments were continued for 21 days. Gravimetric parameters and electrophysiological parameters (Heart Rate, sympatho-vagal balance) were recorded and lipid profiles of all the groups were measured. ANOVA, correlation and multiple regressions were done for analysis of data.

Results: Significant alteration in serum lipid profile was observed in rats fed with high dietary fat but supplementation of EEO was found to be reversible. Electrophysiological evaluation revealed altered HR and sympatho-vagal balance in high dietary fat fed rats(group III) which indicate cardiac autonomic malfunctions which were found to be improved in *Emblica Officinalis* supplemented group of rats (group IV). Further, analysis has shown significant negative correlation between HDL/LDL and sympatho-vagal balance in all groups of rats which clearly indicate a role of dietary fat on sympatho-vagal balance. These results further corroborated with findings of histopathological study on myocardium and elastic artery.

Conclusion: Observations from the study indicate a beneficial role of ethanolic extract of *Emblica Officinalis* (amla) on dyslipidemia and cardiac autonomic functions in rats treated with high fat diet.

Keywords: Atorvastatin High Fat Diet, Lipid Profile, Sympatho-Vagal Balance

INTRODUCTION

Diet containing high fat given to experimental model produces many features of human obesity. Ideally such high fat diet contains a high fraction of saturated fatty acid (83%) and less amount of mono and poly unsaturated fatty acid [1].

Obesity is associated with alternations in plasma lipids and cardio metabolic disorders such as type 2 Diabetes Mellitus, hypertension, non- alcohol fatty liver disorder and sympatho-vagal imbalance. Sympatho-vagal imbalance indicated by depressed heart rate variability (HRV) [2]. HRV is a physiological phenomenon which tells about time interval between heart beats. It is affected by both sympathetic and mainly parasympathetic division of Autonomic nervous system [3].

The new millennium has evidenced the emergence of a modern epidemic; metabolic syndrome with revolting consequences to the health of human worldwide [4]. To reduce the risk associated with high lipid levels, many hypolipidemic drugs and therapies have been developed. Modern drugs such as Statin group are routinely used to combat patients with dyslipidemia which have shown some contraindications. For this reason, relatively safe therapeutic alternative to fight against dyslipidemia including its associated complications is need of the hour.

Amla or *Emblica Officinalis* belongs to Euphorbiacae family possess many active phytochemical components like gallic acid, tannin, flavonoids, emblicanin A and B and ellagic acid [5]. Amla have shown many beneficial effects in variety of diseases like diabetes, eye disorder, scurvy, aging and rheumatism. Beside these affects most of the time amla is used as a cardiac tonic. Being a strong antioxidant amla found to have some influences on regulation of lipid metabolism [6].

The present study was aimed to assess EEO as lipid lowering and cardiovascular protective agent against high dietary fat supplemented to experimental rats.

MATERIALS AND METHODS

The experimental study was conducted in Shri B.M. Patil Medical College, Hospital & Research Centre, Vijayapur, Karnataka, India.

Fresh, mature, healthy and good quality fruits of *Emblica Officinalis* (amla) were procured from the local market, during the months of November 2015 to December 2015.

Preparation of Extract

The dried, coarsely powdered fruit material was extracted with 99% ethanol using Soxhlet apparatus at a temperature below 60^{0}C for 24 hours. The solvent was evaporated under vacuum which gave semisolid mass with respect to the dried powder. This was preserved as stock solution in refrigerator and diluted with distilled water as per required concentration [7].

Animals and Diet

Healthy albino wistar rats (n=30) weighing 180 to 250gms were obtained from animal house of Shri B M Patil Medical college Hospital & Research Centre, Vijayapur, India. All animals were acclimatized for 7 days to the laboratory conditions at 22-24^{0}C and maintained 12 hr. light/dark cycle. All the experimental procedures were performed in accordance with the Institutional Animal Ethical Committee (IAEC) of Shri B. M. Patil Medical College, Hospital & Research Centre, Vijayapur (Dated 7-12-2015). All care was taken for animals during experiment as well as at the time of scarification as per the guidelines of ICMR on animal research [8].

Diet

Normal diet was prepared by keeping protein (casein 18%), carbohydrate (Amylum 60%), fat (vegetable oil 20 %), vitamin and minerals 2%. Subsequently, hyperlipidemic diet was prepared by keeping protein (casein18%), carbohydrate (Amylum 50%), fat (Vegetable oil 30 %), vitamin and minerals 2% [9].

Experimental Protocol

All rats were divided into following five groups with six rats in each group. Group-I served as control (fat 20%), group II fed with ethanolic extract of *Emblica Officinalis* (EEO) for 21 days. Group-III rats fed with high fat diet (fat 30%) for 21 days, group IV-fed with high fat diet (fat 30%) and EEO for 21 days and group V rats were fed with high fat diet (fat 30%) and atorvastatin for 21 days (4mg/kg/day). EEO was diluted in distilled water and administered orally by using force feeding needle with syringe. (100mg/kg b.wt for 21 days). Dietary protocol was maintained on pair feeding [9].

Gravimetry

Estimation of % body weight gain, heart weight and cardio somatic index of albino wistar rats:

The body weight of all rats were recorded at the beginning of experiment (day 1) and on the day of sacrifice (21st day). Heart weight was measured to the nearest of 0.1 mg in a single pan balance (digital weighing machine). Further, cardio somatic index was calculated by the formula heart weight/ total body weight of rats.

Electrophysiology Recoding

HRV recording was done in rats of all groups by using Biopac MP 45. We have recorded heart rate (bpm), sympathetic (LF), parasympathetic (HF) and sympatho-vagal balance (LF/HF).

Sample Collection

All animals were kept for an overnight fast on 21st day. Blood was collected in 10% EDTA tubes by doing retro-orbital puncture. These blood samples were centrifuged at X 2300 G for 10 mins and serum was separated.

Biochemical Parameters: Determination of Lipid Profile: Serum Total Cholesterol (TC), Serum Triglycerides (TG), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and High Density Lipoprotein (HDL) were analyzed by MESPA automated analyzer (Method GOD POD). HDL/LDL was calculated.

Histopathology Procedure

After collecting blood sample, all rats including control were sacrificed by cervical dislocation. The anterior wall of thoracic cage was opened with midline incision. Then heart and elastic artery were carefully collected and isolated immediately and fixed in 10% neutral buffered formalin solution for 24 hours. The fixed tissues were processed routinely and then embedded in paraffin, sectioned to 3–5 µm thickness, deparaffinized and rehydrated using standard techniques. The impact of high fat diet and amla treatment was evaluated by microscopic changes in the architecture of myocardium and elastic artery section, stained with Haematoxyllin & Eosin (H & E) using standard technique.

STATISTICAL ANALYSIS

Values were expressed in terms of Mean ± SD. To determine the significance of inter group differences, One Way ANOVA followed by 'Post Hoc t tests' were done. p≤0.05 was considered statistically significant. Correlation was done between sympatho-vagal balance (LF/HF) and HDL/LDL ratio in all groups of rats by Pearson's correlation. Linear regression was done to predict effect of HDL/LDL ratio on sympatho-vagal balance (LF/HF), by using SPSS software version 16.

RESULTS

Values are expressed as Mean±SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a, b, c, d express significant difference between groups. a depicts comparison with group I, b depicts comparison with group II, c depicts comparison with group III and d depicts comparison with group IV.(*p ≤0.05).

Parameter	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F Value	P value
Initial body weight(1st day) gms	214.3±7.6	207 ±5.5	245.3 ±6.6	221.3 ±7.2	213 ±13	3.2	0.05
Final body weight(21st day) gms	235.3±6.6	224.3±6.6	283.6 ±6.5 $^{a, b}$	241.3 ±7.09 ^{c}	239.6 ±9.5 ^{c}	9.2	0.002*
Percent of body weight gain	8.8 ±0.6	7.6 ±0.9	13.3 ±2.8	8.2 ±0.7	11 ±0.8	2.1	0.155
Heart weight (gms)	1.033 ±0.15	0.8 ±0.2	1.1 ±0.2	1.06 ±0.25	0.9 ±0.1	1.212	0.36
Cardiac-somatic index	0.004 ±0.0005	0.004 ±0.001	0.003 ±0.0005	0.004 ±0.0001	0.003 ±0.0005	1	0.452

[Table/Fig-1]: Effect of *Emblica Officinalis* (amla) on percentage body weight gain, heart weight and cardiac somatic index.

Body weights of rats were significantly increased in group III (high fat fed rats, 30%fats) as compared to group I (control, 20 % fats) and II (EEO supplemented rats) on 21st day. Group IV (high fat fed rats 30%+EEO) and Group V (high fat fed rats30% + atorvastatin) showed significant decrease in final body weight of rats compared to group III rats.

However, per cent body weight gain of rats of group II, IV and V were reduced as compared to group III [Table/Fig-1] .

Heart weight and cardiac somatic index showed no statistical significant differences among all five groups of rats.

Values are expressed as Mean±SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a, b, c, d express significant difference between groups. a depicts comparison with group I, b depicts comparison with group II, c depicts comparison with group III and d depicts comparison with group IV.(*p ≤0.05).

Heart rate levels were significantly higher in rats fed with high fat diet (30% fat, group III) compared with control rats (group I) and rats supplemented with EEO (group II). Also there were significant low values of HR in rats of group IV (high fat fed rats 30% +EEO) & group V (high fat fed rats 30% + Statin) compared with group II.

Sympathetic function (LF) was significantly increased in rats fed with high fat diet (group III) compared to group I (control rats) and group II (EEO supplemented rats). Group IV (high fat fed rats 30% +EEO) and V (high fat fed rats 30% + atorvastatin) have shown significant lower values of sympathetic function (LF) compared to group III.

Parameter	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F Value	P value
HR bpm	131 ± 11	151.6 ± 20	343.6 ±57 a,b	258.6 ±44 c	220.3 ±10 c	18.56	0.000*
Sympathetic (LF)	0.33 ±0.05	0.31 ±0.02	0.59 ±0.09 a,b	0.32 ±0.08 c	0.31 ±0.03 c	10.59	0.001*
Parasympathetic (HF)	0.66 ±0.05	0.66 ±0.01	0.39 ±0.09 b	0.67 ±0.08 c	0.64 ±0.03 c	10.54	0.001*
Symp-vagal balance (LF/HF)	0.5 ±0.01	0.48 ±0.02	1.5 ±0.5 b	0.50 ±0.01 c	0.54 ±0.08 c	10.54	0.001*

[Table/Fig-2]: Effect of *Emblica Officinalis* (Amla) on HRV in all groups of rats.

Parasympathetic function (HF) was significantly lesser in group III compared to group II. There were significantly higher values of Parasympathetic function (HF) in group IV & V compared to group III.

Sympatho-vagal balance (LF/HF) in group III was significantly higher as compared to group II. There was significant decrease of sympatho-vagal balance (LF/HF) in group IV & V compared to group III. [Table/Fig-2].

ANOVA followed by Post Hoc Tukey's multiple comparison test. Superscript a, b, c, d express significant difference between groups. a depicts comparison with group I, b depicts comparison with group II, c depicts comparison with group III and d depicts comparison with group IV.(*p ≤0.05).

TC level was higher in group IV (high fat fed rats 30% +EEO) compared with group II (EEO supplemented rats).Group V (high fat fed rats 30% + atorvastatin) rats have shown significant decrease in levels of TC compared to group III (high fat fed diet 30%) and IV.

Levels of TG were higher in group III compared to group I & II. Group IV and V have shown significantly lesser values for TG compared to group III.

LDL levels were higher in group III compared to group I. VLDL levels have shown significantly high levels in group III compared to group I & II. There was significant decrease in VLDL levels in group V (high fat fed rats 30% + atorvastatin) compared to group III rats.

HDL levels were not significant in all groups although there were higher levels of HDL in group II, IV and V compared to group III

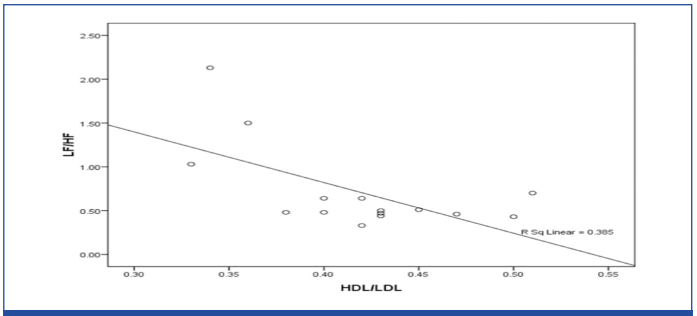
HDL/ LDL ratio was significantly lower in group III (high fat fed rats 30%) compared to group II (rats supplemented with EEO) [Table/Fig-3].

(n=30; r= -0.620, p<0.05*) [Table/Fig-4]: Correlation between sympatho-vagal balance (LF/HF ratio) and HDL/LDL ratio in all groups of rats.

Parameter	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F Value	P value
TC mg/dl	118.6 ± 3.05	114.6 ± 3.05	122.6 ±4.7	120± 4.5 b	110.3 ± 5.5 c,d	6.94	0.006*
TG mg/dl	92.6 ± 4.9	101±8.1	139.6 ± 4.04 a,b	121.3 ± 9.07 c	101.6 ±7.2 c,d	22.3	0.000*
LDL mg/dl	69.2 ±7.9	77 ± 2.5	84.73 ±2.9 a	71 ± 8.5	74.2 ± 1.4	3.62	0.045*
VLDL mg/dl	19.1 ± 1.1	21 ± 2.5	36.1 ± 7.4 a,b	26± 6.2	21 ± 2.5 c	5.65	0.012*
HDL mg/dl	31.6 ± 1.5	35 ± 1	29.3 ±1.1	33.3 ± 4.9	31.3 ±1.5	2.19	0.143
HDL/ LDL ratio	0.44 ±0.05	0.45 ± 0.02	0.34 ± 0.01 b	0.43 ±0.06	0.41 ±0.01	3.56	0.04*

[Table/Fig-3]: Effect of *Emblica Officinalis* (Amla) on Lipid Profile in all groups of rats.

The respective correlations between sympatho-vagal balance (LF/ HF ratio) and HDL/ LDL ratio among all groups of rats have been given in [Table/Fig 4]. [Table/Fig 4] depicted significant negative relationship between sympatho-vagal balance (LF/HF ratio) and HDL/ LDL ratio among all groups of rats.



[Table/Fig-4]: Correlation between sympatho-vagal balance (LF/HF ratio) and HDL/LDL ratio in all groups of rats.

A regression was run to predict sympatho-vagal balance (LF/ HF) from HDL/LDL in all groups of rats. HDL/LDL variable indicates significant effect on sympatho-vagal balance (LF/ HF) [Table/Fig-5].

Superscript 'a' indicates Predictors at constant: HDL/LDL

Here (LF/HF) sympatho-vagal balance (dependent variable; R) with value of 0.620 indicates a good level of prediction. R^2 indicates coefficient of determination. From [Table/Fig-6], R^2 with value 0.385 indicates that our independent variables explain 38.5% of the variability of dependant variable.

[Table/Fig-6] shows that the independent variables significantly predict dependent variable F (1, 13)=4.919 p<0.05*. The regression model is good fit for the data. Regression equation can be formed,LF/HF = 3.133 - 5.782 X HDL/LDL

Model	R	R Square	Adjusted R Square	Std. Error of The Estimate
1	.620a	.385	.337	0.39866

[Table/Fig-5]: Model Summary; multiple linear regressions using HDL/LDL as predictors for sympatho-vagal balance (LF/ HF) in all groups of rats.

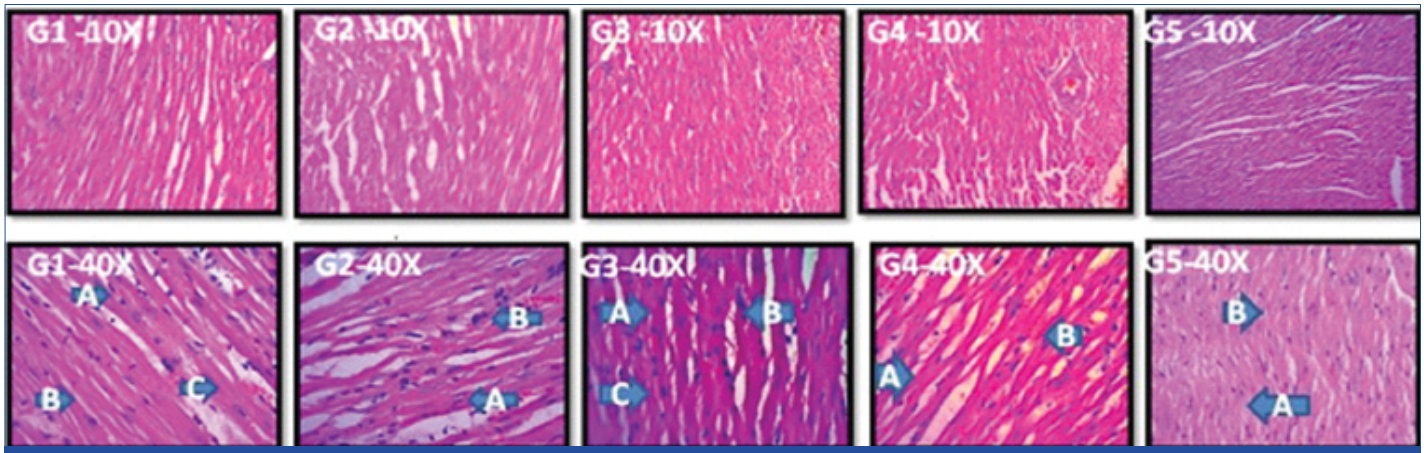
Model	Sum of Squares	df	Mean Square	F	Sig.
1 Regression	1.292	1	8.128	4.919	0.014a
Residual	2.066	13			
Total	3.358	14			

[Table/Fig-6]: Shows that the independent variables significantly predict dependent variable (ANOVA).

Microscopic structure of myocardium in all groups are stained with H & E (10 x & 40x) depicted in two rows. Control group (G1): A central placed nucleus, B intercalated disk, branching and anastomosing striated muscle and C capillary. Group 2 (G2) is rats fed with EEO. A Normal myocardium , B Prominent and central placed nucleus. Group 3 (G3) is rats fed with high fat diet A: myocardial hypertrophy with multiple nucleus, B degeneration & C capillary congestion. Group 4 (G4) is rats fed with high fat diet with EEO A Normal myocardium. B Mild capillary congestion. Group 5 (G5) is rats fed with high fat diet with Statin. A Normal myocardium and B. Prominent and central placed nucleus.

Histopathological architecture of myocardium showed prominent intercalated discs and centrally placed nucleus in control group. There was a mild focal myocardial hypertrophy and degeneration and capillary congestion in group III. And there was no evidence of focal myocardial hypertrophy, degeneration and capillary congestion in treated groups i. e II, IV & V [Table/Fig-7].

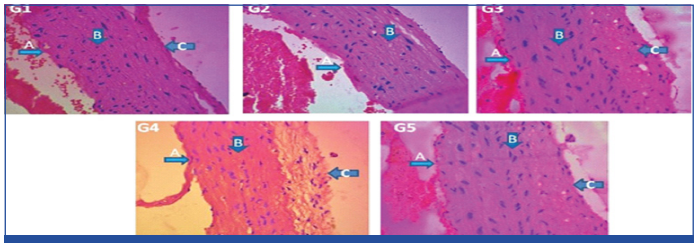
Microscopic structure of elastic artery in all groups are (stained with H & E, in40x) depicted. Control group (G1): A central placed nucleus, B intercalated disc, branching and anastomosing striated muscle and C capillary. G2 is rats fed with EEO: A Normal myocardium , B Prominent and central placed nucleus. G3 is rats fed with high fat diet A: myocardial hypertrophy with multiple nucleus, B:



[Table/Fig-7]: Histopathology of myocardium in all groups of rats.

degeneration & C: capillary congestion. G4 is rats fed with high fat diet with EEO: A Normal myocardium , B Mild capillary congestion. G5 is rats fed with high fat diet with Statin A Normal myocardium and B. Prominent and central placed nucleus.

Microscopic architecture of elastic artery of all the three layers ie tunica intima, media and adventitia showed normal in group I. Rats fed with high fat diet (group III) were presented with morphological alterations in the nuclei of smooth muscle cell of tunica media. The tunica media showed degeneration in round shape and hyperplasia of the smooth muscle cell with round nuclei also elastic fibers showed moderate thickening. The microscopic architecture of tunica intima was lined by endothelial cells showed normal but mild thickening in treated groups II, IV and V. Also tunica media and adventitia were



[Table/Fig-8]: Histopathology of elastic artery.

normal in treated groups II, IV and V [Table/Fig-8].

DISCUSSION

In the present study ethanolic extract of *Emblica Officinalis* was tested for its cardio- protective activity in albino rats fed with hyperlipidemic diet. The degree of protection was assessed by using markers like lipid profile, HRV including sympathetic (LF), parasympathetic (HF) and LF/ HF ratio along with histopathological study of cardiac tissue.

Our results indicate that hyperlipidemic diet has shown an adverse effect on total body weight of rats. Subsequently, increase in % of body weight gain of rats fed with high fat diet. We observed loss of body weight in rats after administration of amla indicates the interference of protein metabolism.

In hyperlipidemic rat model, amla has shown significant changes in lipid profile may be due to several mechanisms such as an interference with cholesterol absorption [10]. Flavonoids present in amla significantly lowered lipid levels in serum and tissues by its inhibitory action on HMG CO-A reductase pathway [11].

Also, standardized extract of amla fruit increases cardiac glycogen levels and decreases LDL levels. The mechanism may include increase in LCAT (Lecithin Cholesterol acyltrans): the enzyme responsible for ester transfer to HDL. Similarly, there may be increase in lipoprotein lipase activity. This increased enzymatic activity increases the ability of muscle fibers to oxidize fatty acids coming from VLDL, TG. This process of cholesterol removal from circulation is termed as reverse cholesterol transport [12].

Mand et al., observed in their study, decrease in total cholesterol levels in hypercholesterolemia rabbits when they were given amla for 12 weeks. Similar results were observed in present study [13]. Our report further corroborated with previous findings that showed a significant decrease in TC, LDL, TG and significant increase in HDL levels in amla treated hyperlipidemic rats [6].

It is reported that Polyphenols present in amla fruit juice (541.3 mg gallic acid equivalent / 1 gm extraction) might be responsible for lipid lowering effects of fruit juice which may explore cardioprotective effects [14].

Roy A et al., showed that hypercholesterolemia is associated with decreased 24 hour heart rate variability. Decreased heart rate variability is a valuable predictor of CHD [15]. Our findings support these results.

It has been reported that rats on hyperlipidemic, hypercholesterolemia diet in comparison to the control group showed significant increase in HR, MBP. HR is an indicator of sympathetic nervous system. Sympathetic nervous system activity increases with fat induced calorie intake [16].

Previous report suggested that an improvement in the autonomic functions (sympatho-vagal balance LF/HF) in Statin associated therapy in CHD and hyperlipidemea among general population. Our results support these findings with higher LF/HF [17].

It has been reported that atherogenic ratio was significantly increased in high fat diet induced hyperlipidemic rats compared to control rats. There was significant decrease in atherogenic ratio in hyperlipidemic rats treated with polyherbal formulation (OB- 6). [Polyherbal formulation (OB- 6) is extracts of six medicinal plants *Cassia angustifolia, Nigella sativa, Phyllanthus amarus, Emblica officinalis, Zingiber officinale* and *Terminalia chebula*] which corroborates with our findings [18].

Ojha S et al., showed beneficial effects of amla on haemodynamic parameters as well as improvement in left ventricular functions in Isoproterenol-induced MI rats [19].

We observed marked structural changes in histopathology of myocardium in rats of group III (rats fed with high fat diet) which has shown a mild focal myocardial hypertrophy, degeneration and capillary congestion whereas, a remarkable improvement in myocardial architecture in rats of group IV (rats fed with high fat diet with EEO). Similarly, we observed normal myocardium in rats of group II (rats treated with EEO) and V (rats fed with high fat diet treated with Statin). This indicates cardioprotective effect of amla on myocardium by preserving its normal architecture via its antioxidant property.

From the results it is clear that EEO treated group showed beneficial effects on the lipid profile and electrophysiological aspects in

the study. This could be due to the nutrient and phytochemical composition of fruit of amla which has a high antioxidant values. Probably active compounds like tannins, gallic acid, emblicanin and ascorbic acid might have played a protective role in cardiovascular pathophysiology on experimental animals.

LIMITATION

Present study on *Emblica Officinalis* was done with phytochemical extract but active biological compound of *Emblica Officinalis* was not assessed.

CONCLUSION

Results clearly indicate that ethanolic extract of *Emblica Officinalis* (amla) protect against high dietary fat induced dyslipidemia in experimental animals. Results further conclude that ethanolic extract of *Emblica Officinalis* (amla) might have cardioprotective actions in hyperlipidemic rats by modulating cardiac autonomic functions.

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