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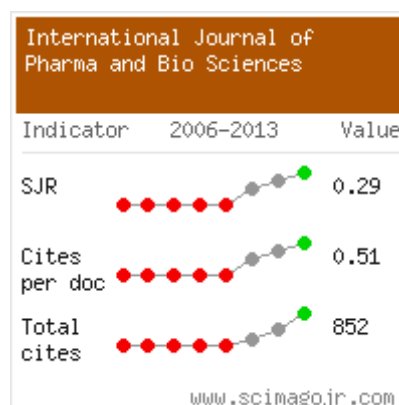
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## EFFECT OF ETHANOLIC EXTRACT OF *EMBLICA OFFICINALIS* (AMLA) ON PATHOPHYSIOLOGY OF LIVER IN HYPERLIPIDEMIC ALBINO WISTER RATS.

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### ABSTRACT

It has been reported that hyperlipidemia plays central role in the development of atherosclerosis, liver disorders and oxidative stress. *Embilica officinalis* also known as Amla or Indian Gooseberry acts as antihyperlipidemic, antioxidant and liver tonic. It actively contains tannins, gallic acids and flavonoids. Aims: To evaluate the effect of the Ethonolic extract of *Embilica Officinalis* on pathophysiology of liver and on biochemical parameters in Hyperlipidimic albino Wister rats. Extraction of *Embilica Officinalis* by Soxhlet apparatus using 99% ethanol at 60° temp for 24hrs and Phytochemical analysis was done. Group I served as normal control. Group II Fed with Isocaloric diet. Group III Fed with Hyperlipidimic diet. Group IV. (Isocaloric diet 21 days + *Embilica Officinalis* 21 days). ok(hyperlipidemic diet 21 days+ *Embilica Officinalis* 21 days). Dose of ethonolic extract of *Embilica Officinalis*: (100mg/kg b. wt daily). %body weight gain, liver weight and hepatosomatic index were significantly improved in hyperlipidemic rats treated with Amla. There was significant improvement in lipid profile and markers of liver functions. Liver shown fatty changes in hyperlipidemic rats and normal Hepatocytes in Hyperlipidimic rats treated with Amla. It can be concluded that amla may be effective , natural therapeutics in hyperlipidemia and liver disorders.

**KEYWORDS:** *Embilica Officinalis*, Hyperlipidimic diet, Histopathology of Fatty Liver, LFT, Lipidprofile



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## INTRODUCTION

According to the model texts Charak Samhita and Shushruta Samhita *Embilica Officinalis* regarded as "One of the best rejuvenating herbs".<sup>1</sup> The fruits of *Embilica Officinalis* are used as dietary and medicinal purposes by Indian system of medicine.<sup>2</sup> It is commonly known as Amla or Indian gooseberry. The major principles of *Embilica Officinalis* are hydrolyzable tannins (embilica A & B), Gallic acid flavonoides, flavones and ascorbic acid. The *Embilica Officinalis* exerts various biological functions such as antioxidant, anti-atherosclerosis, antidiabetic, hypolipidemic, gastroprotective and cytoprotective. Along with these functions *Embilica Officinalis* also produce beneficial effects on liver functions As well as alleviate hyperlipidemia and metabolic syndrome.<sup>3</sup> As liver is the major organ of metabolic and energy homeostasis. Its balanced actions are over levels of endogenous metabolites such as TG, TC, HDL and glucose.<sup>4</sup> The hepatoprotective actions of *Embilica Officinalis* noticed to be mediated by its free radical scavenging, antioxidant and modulation of lipid metabolism.<sup>2</sup> In the present study we tried to or is it to prove? the effect of *Embilica Officinalis* on pathophysiology of liver of hyperlipidemic rats. We induced hyperlipidemic diet to the albino wister rats to develop an animal model of metabolic syndrome expressing fatty liver and other cardiovascular risk factors. Hyperlipidemic diets generate atherosclerosis, changes in lipids and hepatic steatosis.<sup>5</sup> Atherosclerosis is a disease that involves the interplay of several factors like oxidation of lipoproteins, formation of atherosclerotic plaques.<sup>6</sup> Amla like statins act to inhibit HMGCoA reductase activity and ellagic acid acts to inhibit cholesterol biosynthesis.<sup>7</sup> Hence the present study investigated the therapeutic efficacy of *Embilica Officinalis* extract on pathophysiology of liver of hyperlipidemic albino wister rats.

## MATERIALS AND METHODS

### Materials

Fresh, mature, healthy and good quality fruits of *Embilica Officinalis* (Amla) were procured from the local market, during the months of November–December 2012 identified and

authenticated. Ethonolic extract preparation: 300gms of the powder of dry fruits of *Embilica Officinalis* was extracted with 99% ethanol using Soxhlet apparatus at a temperature below 60° C for 24 hours. The solvent was evaporated under vacuum which gave semisolid mass with respect to the dried powder.

### Experimental Animals

Albino wister rats weighing 180 to 250gms were obtained from animal house of Shri B M Patil Medical college Hospital & Research Centre, Bijapur. All the five group animals were acclimatized for 7 days to the laboratory conditions at 22-24°C and maintained 12 hr. light/dark cycle All the experimental procedures were performed in accordance with the approval of the Institutional Animal Ethics Committee (IAEC) of Shri B M Patil Medical college Hospital & Research Centre, Bijapur. All care was taken for animals during experimental as well as at the Time of sacrifice as per the guidelines of ICMR on animal research Reference?

### Experimental protocol

All the rats were divided into following five groups with 6 rats in each group. Group-I, Fed with water and ad libitum serve as control, Group II Fed with Isocaloric diet for 42 days Group-III Fed with high fat diet 42 days, Group IV fed with Ethanolic extract of *Embilica Officinalis* (EEO) (21 days Isocaloric diet + 21 days with EEO) and Group V Fed with high fat diet and EEO Ethonolic extract of *Embilica Officinalis* (21 days high fat diet + 21 days with EEO). It was given daily 100mg/kg B.Wt, I.P.

### Sample collection and Tissue collection

All the five group animals were sacrificed by cervical dislocation at the end of the last dose after an overnight fast. After heart puncture blood was quickly collected in 10% EDTA tubes for the separation of serum. Tissue collection for histopathology: liver was 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  $\mu\text{m}$  thickness, deparaffinized, and rehydrated using standard techniques. The extent of Hyperlipidemic diet-induced necrosis and steatosis was evaluated by assessing morphological changes in liver sections stained with hematoxylin and eosin (H&E), using standard techniques.

### Gravimetry

#### **Estimation Body Weight and Hepatosomatic Index of Albino Wister rats**

The body weight of all rats was recorded at the beginning of experiment (day 1), treatment with *Emblica Officinalis* (21<sup>st</sup> day) and on the day of sacrifice (42<sup>nd</sup> day). Liver weight was measured to the nearest of 0.1 mg in a single pan balance (Digital weighing machine). Further hepatosomatic index was calculated by the formula liver weight/ total body weight.

#### **Determination of Hematological Parameters**

Hb%, RBC, WBC, Platelet, PCV & MCHC these Hematological parameters like Hb% and MCHC were evaluated in were evaluated in Sysmax -21, automated analyzer.

### Biochemical Analysis

#### **Estimation of Lipid profile**

Serum triglycerides (TG), Serum total cholesterol (TC), High-density lipoprotein (HDL), Low-density lipoprotein (LDL) and Very Low-density lipoprotein (VLDL) were analyzed by MESPA automated analyzer (Method GOD POD)

#### **Estimation of Liver Function Tests**

Serum Glutamic Oxalocetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Serum Protein, Serum bilirubin, Serum albumin, Serum A/G Ratio and Serum Alkaline Phosphatase (ALP) were analyzed by Meril diagnostic Kit Method.

### STATISTICAL ANALYSIS

Values are expressed as Mean  $\pm$  SD. To determine the significance of inter group differences, One Way ANOVA followed by 'Post Hoc t tests' were done.  $P \leq 0.05$  was considered statistically significant.

## RESULTS

Table 1

**Effect of Ethanolic extract of *Emblica Officinalis* (Amla) on % body weight gain (on 21<sup>st</sup> day and 42<sup>nd</sup> day) on different groups of rats.**

% body weight gain	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F value	P value
At 21 <sup>st</sup> day	21 $\pm$ 1.7	14.7 $\pm$ 4.8 <sup>a</sup>	17.5 $\pm$ 4.5	21.6 $\pm$ 1.2 <sup>b</sup>	15.6 $\pm$ 2.2 <sup>d</sup>	5.331	0.003
At 42 <sup>nd</sup> day	15.6 $\pm$ 0.7	14.7 $\pm$ 2.4	17.1 $\pm$ 2.4	8.6 $\pm$ 2.6 <sup>a,b,c</sup>	8.8 $\pm$ 3.4 <sup>a,b,c</sup>	17.6	0.00

Values are expressed as mean $\pm$ 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. (\*  $\leq 0.05$ ). Group I is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is ethanolic extract of Amla fed rats, Group V is hyperlipidemic + Ethanolic extract of Amla

#### **Table 1**

##### **At 21<sup>st</sup> day**

We observed significant decrease in % body weight gain in group II compared to group I. Group IV showed significant elevation in % body weight gain compared to group II. There was significant decrease in % body weight gain in group V compared to group IV.

##### **At 42<sup>nd</sup> day**

Group IV and V depict significant decrease in % body weight gain compared to group I, II and III.

**Table 2**  
**Effect of Ethanolic extract of Embilica Officinalis (Amla) on Liver weight and Hepatosomatic index of different groups of rats.**

Parameters	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F Value	P value
Weight of Liver	7.73±0.17	8.87±0.95	6.72±0.36	7.67±0.22	6.68±0.83	2.246	0.093
Organosomatic Index	0.03±0.00	0.03±0.00	0.02±0.00	0.03±0.00	0.02±0.00	1.416	0.258

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. (\* ≤0.05). Group I is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is ethanolic extract of Amla fed rats, Group V is hyperlipidemic + Ethanolic extract of Amla

**Table 2**

depicts no statistical significant differences of liver weight and Hepato somatic index among all five groups of rats.

**Table 3**  
**Effect of Ethanolic extract of Embilica Officinalis on Heamatological parameters in different groups of albino wistar rats.**

Parameters	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F value	P value
Hb%	12.8±1.1	12.9±1.2	12.8±0.5	13.3±0.14	12±0.8	1.682	0.18
WBC	7783±2806	7266±2084	5633±1723	7383±2918	6566±1538	0.82	0.52
RBC	7.4±0.9	7.3±0.8	7.3±0.3	8±0.2	6.8±0.3 <sup>d</sup>	2.667	0.03
Platelet	8.1±2.6	6.4±2.2	7.6±1.2	9.7±0.3	8.2±2.7	2.041	0.11
PCV	44.6±5.4	43.6±4.8	42.1±2.3	47.9±1.9	39.8±2.5 <sup>d</sup>	3.895	0.01
MCHC	28.9±1.3	29.6±0.6	30.5±0.8	28.1±1.1 <sup>c</sup>	30.3±1.7 <sup>d</sup>	4.286	0.009

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. (\* ≤0.05). Group I is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is ethanolic extract of Amla fed rats, Group V is hyperlipidemic + Ethanolic extract of Amla

**Table 3**

Values of Hb%, WBC and platelet have shown statistically non significant differences among all groups. RBC and PCV were significantly lower in group V compared to IV. We observed no significant relation for RBC and PCV

between groups III and V. MCHC value has shown significantly higher values in group V compared to IV. Also there was significantly lower MCHC value in group IV compared to group III.

**Table 4**  
**Effect of Ethanolic Extract of Embilica Officinalis on lipid profile parameters in different groups of rats.**

Parameters	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F value	P value
TG mg/dl	160.1±20.8	140.3±35.9	200.3±93.2 <sup>a</sup>	110±23.5 <sup>c</sup>	129±32.2	3.60	0.01
TCmg/dl	130.6±13.6	122.8±16.1	123.1±17.5	131.5±15.8	185±17.1 <sup>a,b,c,d</sup>	15.8	0.000
HDLmg/dl	30.3±1.86	30.0±1.89	41.8±14 <sup>a,b</sup>	29.6±1.86 <sup>c</sup>	36.8±2.5	4.08	0.01
LDLmg/dl	80.6±12.5	64.7±18.5	33±15.8 <sup>a,b</sup>	79.8±12.8 <sup>c</sup>	122.3±14.1 <sup>a,b,c,d</sup>	27.94	0.000
VLDLmg/dl	22.7±4.2	28±7.1	44±18.6 <sup>a</sup>	22±4.7 <sup>c</sup>	25±6.7	3.319	0.026

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. (\* ≤0.05). Group I is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is ethanolic extract of Amla fed rats, Group V is hyperlipidemic + Ethanolic extract of Amla.

**Table 4**

Levels of TG were significantly higher in group III compared to group I. Group IV showed significant decrease in TG levels compared to group III. No significant differences were observed for TG levels between group IV and group V. TC levels were significantly higher in group V compared to group I, II, III & IV. We observed significantly higher values of HDL in group III compared to group I and group II. HDL levels showed significantly lower values in group IV compared to group III. No significant differences were observed between group III

and V although there were decreased in HDL levels in group V. LDL levels were significantly lower in group III compared to group I & II. We observed significantly higher values of LDL in group IV compared to group III. Also, there were significantly higher values of LDL in group V compared to Group I, II, III and IV. Group III showed significant higher levels of VLDL compared to group I. It was shown significant decrease in VLDL levels in group IV compared to group III. No significant differences were observed for VLDL between group III and V.

**Table 5**  
**Effect of Ethanolic extract of *Embilica Officinalis* on liver parameters in different groups of albino wistar rats**

Parameters	Group I	Group II	Group III	Group IV	Group V	ANOVA	
						F value	P value
Serum bilirubin mg%	0.83±0.1	0.6±0.01	0.78±0.1	0.8±0.1	0.68±0.19	2.55	0.06
SGOT U/L	51.6±8.98	73.6±6.5	70.1±28.9	51.3±8.1	18.6±5.04 <sup>a,b,c,d</sup>	13.5	0.00
SGPT U/L	48.1±4.83	59.5±19.7	64.5±12.5	47.16±5.67	22.83±4.26 <sup>a,b,c,d</sup>	12.52	0.00
Serum protein gm/dl	5.68 ±0.26	5.71 ± 0.25	5.7 ±0.25	5.6 ± 0.24	6.01±0.38	1.58	0.21
Serum albumin gm/dl	2.9±0.16	2.9±0.1	3±0.19	2.86±0.13	3.4±0.34 <sup>a,b,c,d</sup>	6.44	0.001
A/G ratio gm/dl	0.9±0.08	1±0.10	1±0.11	0.98±0.04	1.30 ±0.24 <sup>a,b,c,d</sup>	6.42	0.001
Alkaline phos U/L	16.3±1.7	15.3±1.6	18.7±5	16.1±1.8	17.2±2.6	1.27	0.30

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. (\* ≤0.05). Group I is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is ethanolic extract of Amla fed rats, Group V is hyperlipidemic + Ethanolic extract of Amla

**Table 5**

Serum bilirubin, serum protein and alkaline phosphate have shown statistically non significant differences among all groups. SGOT, SGPT and serum albumin levels were significantly lower in group V compared to group I, II, III and IV. A/G ratio was significantly higher in group V compared to I, II and IV

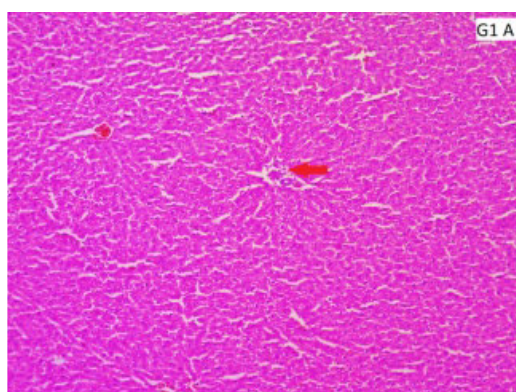
**THE HISTOPATHOLOGY OF LIVER**

Group I histopathology section of liver shown normal hepatic architecture compressed of

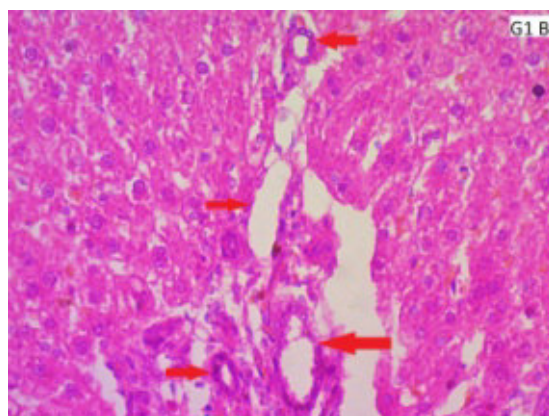
hepatic lobules formed by central vein and cords of hepatocytes with indistinct sinusoidal dilatation but whereas Group II, IV & V has shown prominent sinusoidal dilatation (Fig 1, 2, 4 & 5 H&E A10X and B40X) Group III; histopathology of liver shown lobular architecture of the liver with enlarged hepatocytes containing microvesicular and macrovesicular fatty changes with sinusoidal congestion. (Fig 3 H&E A10X and B40X)



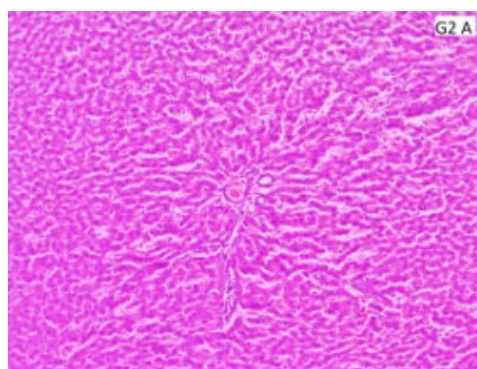
**FIGURES: Showing Histopathology of Liver]**



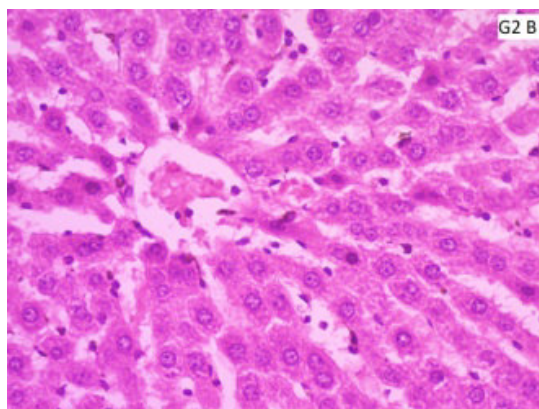
**Figure 1**  
**Group I A. Showing normal Hepatocytes in lobular pattern in 10X**



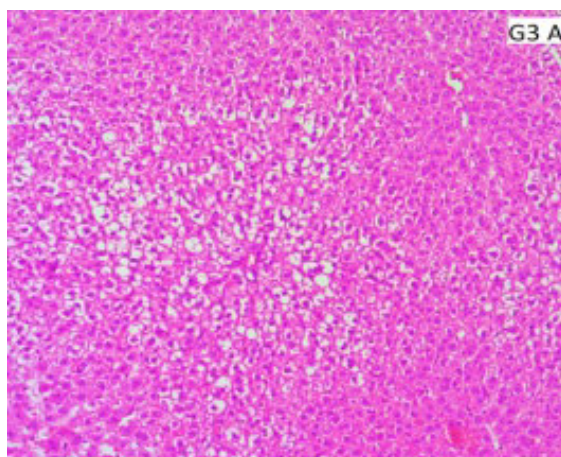
**Figure 2**  
**. Group I. B. Showing normal architecture of liver with portal triad (Bile Duct, Hepatic Artery, & Vein) in 40X**



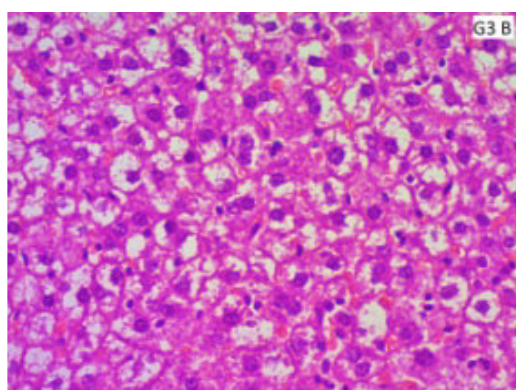
**Figure 3**  
**Group II. A. Showing normal architecture of Liver with Central vein surrounded by Hepatocytes and intervening Sinusoids in 10X.**



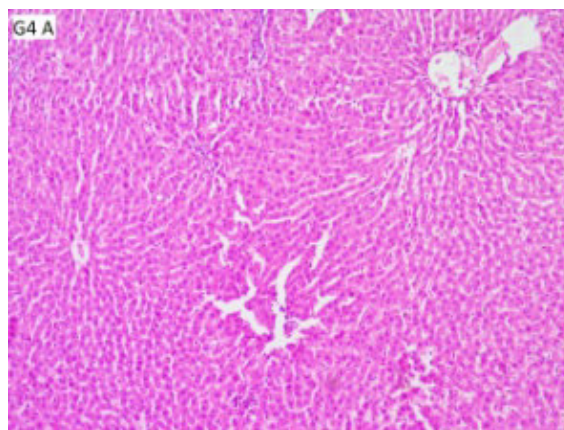
**Figure 4**  
**Group II. B. Showing normal architecture of Liver with Central vein surrounded by Hepatocytes and intervening Sinusoids in 40X**



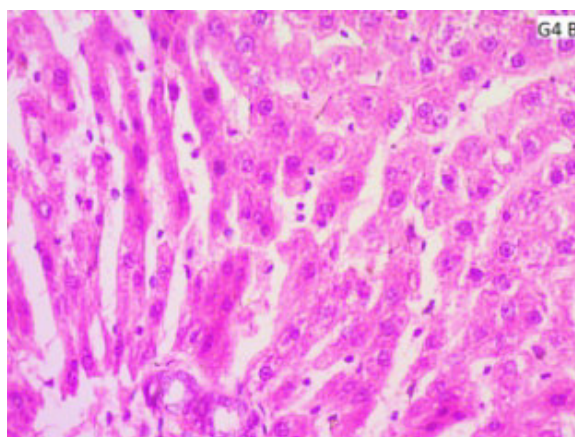
**Figure 5**  
**Group III. A. Architecture of liver showing prominent Macrovesicular & Microvesicular fatty change in 10X**



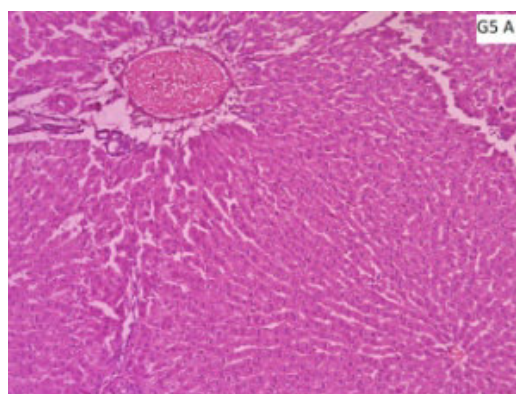
**Figure 6**  
**Group III.B. Architecture of liver showing prominent Macrovesicular & Microvesicular fatty change in 40X**



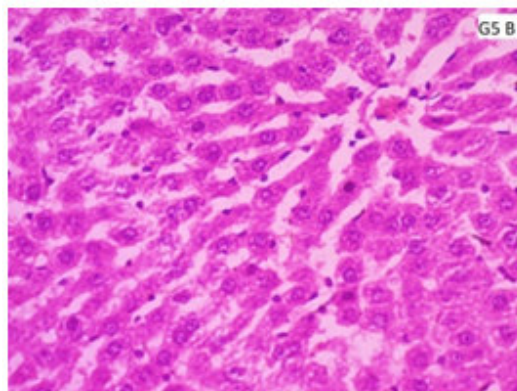
**Figure 7**  
**Group IV A. Architecture of liver showing prominent Hapatocytes separated by dilated sinusoids in 10X**



**Figure 8**  
**Group IV B. Architecture of liver showing prominent Hapatocytes separated by dilated sinusoids in 40X**



**Figure 9**  
**Group V A. Architecture of liver showing Hepatocytes separated by dilated sinusoids in 10X.**



**Figure 10**  
**Group V. B. Architecture of liver showing Hepatocytes separated by dilated sinusoids in 40X**

## DISCUSSION

Animal models offer an appropriate mode to explore and understand the pathophysiology of disease and open the door to prevent or to treat the studied disease. Studies on such models significantly add towards enriching knowledge in the field of medical research. Hyperlipidemic animal models express clinical manifestations of fatty liver and other cardiovascular risk factors. The hyperlipidemic effects of the diet demonstrated in the increased body weight and lipid profile in albino wister rats.<sup>5</sup> And the hyperlipidemic rats treated with *Embilica Officinalis* showed normal body weight, lipid profile as well as normal liver histology. We observed decrease in % body weight gain in rats treated with *Embilica Officinalis* compared to Group I at 21<sup>st</sup> day. Also, there was a significant decrease in % body weight gain in rats treated with *Embilica Officinalis* compared to hyperlipidemic rats and control at 42<sup>nd</sup> day. There was no significant difference observed between hyperlipidemic rats treated with *Embilica Officinalis* and rats treated with only *Embilica Officinalis* at 42<sup>nd</sup> day, this could be due to excessive breakdown of tissue proteins and catabolism of fats and proteins.<sup>8</sup> B Antony et al, reported in their study a significant reduction in TC, LDL, VLDL & TG whereas there was significant elevation in the HDL level after treatment with *Embilica Officinalis*. In addition haemogram showed improved levels of Hb, RBC and other cells.<sup>7</sup> Similar results were

observed in our study. Anju Lama et al showed significant increase in all the lipid parameters ( $p < 0.01$ ) except HDL following administration of high fat diet. It was also seen that administration of the EEO at a dose of 1 gm/kg body weight along with high fat diet in the experiment animals, showed a significant decrease in all the lipid parameters ( $p < 0.01$ ) with a significant rise in the value of HDL ( $p < 0.01$ ).<sup>6</sup> We found a significant increase in levels of TC and LDL in group V (hyperlipidemic rats treated with Amla) compared to groups I, II, III and IV. SGOT & SGPT are definitive indicators of liver parenchymal injury. The enhanced levels of plasma SGOT and SGPT may be due to the leakage of these enzymes from liver cytosole to the blood stream which is the marker of hepatic toxicity.<sup>9</sup> Manik K Singh et al, reported no significant effect on SGOT and SGPT levels in mice treated with Amla as compared to control. In contrast, we observed significant decrease in levels of SGOT and SGPT in group V (Hyperlipidemic rats treated with Amla) compared to group I, II, III and IV.<sup>10</sup> Rupal A Vasant et al observed significantly reduced plasma ALP levels in the rats treated with Amla ( FEO 2.5, FEO 5, FEO 10) compared to normal control and fluoride control groups. In our study, we have shown no significant plasma ALP levels in group V (Hyperlipidemic rats treated with Amla) compared to group I, II, III and IV.<sup>11</sup> In our study, histopathological

analysis at the end of 3 weeks showed increased fat deposition in liver of rats fed with hyperlipidemic diet Group II. Similar observation was reported by Jasmine Bathera et al in their study. They showed hepatocellular fat deposition and ballooning in the liver samples of high fat fed hamsters compared to control hamsters. Control hamsters showed normal liver histology.<sup>5</sup> Jitendra Kumar et al, reported significant decrease in albumin concentration in Amla treated chickens compared to control. In contrast, our study has shown significant increase in serum albumin in group V (Hyperlipidemic diet + Amla treatment) compared to all four groups. They have also shown significant increase in levels of serum protein and non significant reduction of A/G ratio in Amla treated group as compared to control group.<sup>12</sup> Whereas our study has shown significant increase in A/G ratio in group V compared to group I, II and IV. The liver samples of Group III Hyperlipidemic rats showed the lobular architecture with enlarged Hepatocytes containing microvesicular and macrovesicular fatty changes with sinusoidal congestion in (Fig 5. Group III A. H&E 10X, Fig 6 group 3.B H&E 40X). Administration of Hyperlipidemic diet with *Emblica Officinalis* showed near normal appearance of Hepatocytes (Fig. 9. Group V A H&E 10X, Fig 10. Group V.B. H&E 40X). Similar observation was found in V. Damodara Reddy et al study.<sup>13</sup> Although the exact mechanism by which amla exerts this beneficial effect is presently not clear, it brings about favorable changes in the lipid profile via several mechanisms, including interference with cholesterol absorption, inhibition of HMG-CoA reductase activity, and an increase in lecithin cholesterol acyl

transferase activity.<sup>14</sup> The mechanism by which *Emblica Officinalis* exerts its beneficial effects may be like Statins. *Emblica Officinalis* is containing phenolic groups like Tannins, Gallic acid which act like Statin. Like Statin, *Emblica Officinalis* inhibits HMG CoA reductase activity. Ellgitannins and Ellagic acid obtained on hydrolysis of tannins inhibits epoxidase enzyme, a rate limiting enzyme of cholesterol biosynthesis.<sup>7</sup> *Emblica Officinalis* contains many liver tonic which can be used against acute viral hepatitis and other liver disorders.<sup>15</sup> In our study we tried to investigate positive influence of *Emblica Officinalis* on pathophysiology of liver of hyperlipidemic albino wister rats. We observed *Emblica Officinalis* useful in regulating hyperlipidemia and pathophysiology of liver in albino wister rats fed with hyperlipidemic diet.

## CONCLUSION

It can be concluded from our study that *Emblica Officinalis* may be good, natural potential therapeutics for Dyslipidemia and hepatic dysfunction. However extensive clinical studies are required in large numbers of patients to establish the efficacy and safety of *Emblica Officinalis* in the management of Dyslipidemia and related disorders like atherosclerosis and hepatic dysfunction.

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