EFFECT OF ETHANOLIC EXTRACT OF TERMINALIA ARJUNA ON LIVER FUNCTIONS AND HISTOPATHOLOGY OF LIVER IN ALBINO RATS FED WITH HYPERLIPIDEMIC DIET

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ABSTRACT

Objective: The aim of the present study was to assess the effect of Ethanolic extract of Terminalia Arjuna on Liver functions, Lipid profile and histopathology of liver of albino rats fed with hyperlipidemic diet.

Methods: Extraction of Terminalia arjuna bark by Soxhlet apparatus using 99% ethanol at 60 ° temp for 22 h and Phytochemical analysis was done. Dose of Ethanolic extract of Terminalia arjuna: (500 mg/kg Body weight daily).

Results: %body weight gain and hepatosomatic index were significantly improved in hyper lipidemic rats treated with Terminalia arjuna. There was significant improvement in markers of liver functions. Liver shown microvesicular and macrovesicular fatty changes in hyper lipidemic rats and normal Hepatocytes in Hyperlipidemic rats treated with Terminalia arjuna.

Conclusion: It can be summarized that Terminalia arjuna is good, natural therapeutics in hyperlipidemia and liver disorders.

Keywords: Terminalia arjuna, Hyperlipidimic diet, Histopathology of Fatty Liver, LFT and hepatosomatic Index.

INTRODUCTION

This is the speed changing world of extreme disparity like over nutrition and starvation, lifestyle changes and its related diseases, development and environmental degradation. The modern system of medicine is no exception. It cures one hand and trigger side effects on the other [1]. There is increasing demand of people towards an Ayurvedic system of medicines which shows reduced adverse effects on health.

Among many precious herbal drugs Terminalia arjuna holds the pride place in the reference of such medicinal value. Terminalia arjuna is a deciduous and green tree belongs to Combretaceae family, also known as Arjuna or Arjun tree. Terminalia arjuna tree is about 60 to 80 feet in height and it is found in Indo-sub Himalayan tracts of Uttar Pradesh, southern Bihar, Barma, Madhya Pradesh and Duccan region. It grows almost in all types of soils, but prefers humid, fertile loam and karghaic soils.

The bark gets flaked off itself in the month of April-May[2] Its stem bark has active principles like glycosides, flavonoids, tannins and minerals [3]. Flavonoids acts like antioxidants, anti-inflammatory and lipid lowering effect where as glycosides are cardio tonic. It also has hepatoprotective effect [4].

The liver is the primary organ which plays an important role in metabolic and excretion and maintains homeostasis of the body[5] Hyperlipidemia is greatest risk factors for prevalence and severity of coronary heart diseases [6] Hyperlipidemia is also one of the major causes of liver injury. Management of liver diseases still challenges to the modern system of medicine.

Terminalia Arjuna is capable of protecting the liver against hyperlipidaemia, oxidative stress and/or toxin effects [7].

Hence the present study was aimed to assess the effect of Ethanolic extract of Terminalia arjuna on liver in albino rats fed with hyperlipidemic diet. The effects were evaluated by measuring the levels of the serum marker enzymes followed by liver histo pathology.

MATERIALS AND METHODS

Materials: Fresh, bark of Terminalia arjuna was procured from the herbal garden of Ayurvedic medical college, during the months of Nov-Dec 2012 identified and authenticated by Department of Botany KCP Science College Bijapur

Extraction of drug

Ethanolic extract preparation: 250 gms of the powder of the dry bark of Terminalia arjuna were extracted with 99% ethanol using a Soxhlet apparatus at a temperature below 60 °C for 22 h. The solvent was evaporated under vacuum which gave semisolid mass with respect to the dried powder [8].

Phytochemical screening

Terminalia arjuna was screened for the presence of phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrates by using standard protocols [9].

Experimental animals

Albino Wistar rats weighing 160 to 250 gms were obtained from animal house of Shri B M Patil Medical College Hospital and Research Centre, Bijapur. All the four group animals were acclimatized for 7 d 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 and Research Centre, Bijapur. All care has taken on animals during experimental as well as at the time sacrificed as per the guidelines of ICGR on animal research 2006

Experimental protocol

All the rats were divided into following four groups with 6 rats in each group. Group-I, Fed with water and ad libitum serve as a control, the Group II Fed with isocaloric diet for 42 d Group-III Fed with high fat diet 42 d, the Group IV Fed with high fat diet and Ethanolic extract of Terminalia arjuna (21 d high fat diet+21 d with...
Ethonolic extract of *Terminalia arjuna*. It was given daily 500 mg/kg Body Weight, I.P [10].

**Preparation of isocaloric diet**

For 1 kg of diet, 180 gm of casein, 620 gm of carbohydrate, 200 gm of fat and 1% of multivitamin and 2% NaCl was taken [11].

**Preparation of hyperlipidemic diet**

For 1 kg of diet, 180 gm of casein, 520 gm of carbohydrate, 300 gm of fat and 1% of multivitamin and 2% NaCl was taken [11].

**Sample collection and tissue collection**

All the four group animals were sacrificed by cervical dislocation at the end of the last dose after an overnight fast. After heart puncture blood was collected in normal tubes for the separation of serum.

Tissue collection for histopathology; liver was isolated immediately and fixed in 10% neutral buffered formalin solution for 24 h. The fixed tissues were processed routinely, and then embedded in paraffin, sectioned into 4-5 µm thickness, deparaffinized, and rehydrated using standard techniques. The extent of Hyperlipidimic 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 Hepato-somatic Index of Albino Wistar rats:

The body weight of all rats was recorded at the beginning of the experiment (day 1), treatment with an Ethanolic extract of *Terminalia arjuna* (21st day) and on the day of sacrifice (42nd day). Liver weight was measured to the nearest of 0.1 mg in a single pan balance (Digital weighing machine). Further, we calculated Hepato-somatic index by the formula liver weight/total body weight.

**Biochemical analysis**

In biochemical analysis, we estimated lipid profile and liver function test

**Estimation lipid profile**

Serum triglycerides (TG), Total Cholesterol (TC), High density lipoprotein (HDL), Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) were analysed by the MESPA automated Analyzer.

**Estimation of liver function tests**

Serum Bilirubin, total, Serum Glutamic Oxaloctic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Serum Protein, Serum bilirubin, Serum albumin, Serum A/G Ratio and Serum Alkaline Phosphatase (ALP) analysed by Meril diagnostic Kit Method.

**Statistical analysis**

Values are expressed as mean±SD. To determine the significance of intergroup differences, One Way ANOVA followed by ‘Post Hoc t tests’ by SPSS software was done. P≤0.05 was considered statistically significant.

**RESULTS**

We observed a significant increase in levels of TG and VLDL in group 3 (Hyperlipidemic diet fed rats) compared to group 1 (Normal control rats). Group 4 (hyperlipidemic rats treated with ethanolic extract of *Terminalia arjuna*) rats showed a significant increase in levels of TC and LDL compared to Group 1, 2 and 3 respectively. HDL levels showed a significant increase in group 3 (Hyperlipidemic diet fed rats) compared to groups 1 and 2 respectively. We also observed significant decrease in LDL levels in group 3 (Hyperlipidemic diet fed rats) compared to groups 1 and 2 respectively.
Fig. 5: G3 10X HandE stain. architecture of liver showing prominent microvesicular and macrovesicular fatty change

Fig. 6: G3 40X HandE stain architecture of liver showing prominent microvesicular and macrovesicular fatty change

Fig. 7: G4 10X HandE stain architecture of liver showing prominent hepatocytes separated by dilated sinusoids

Fig. 8: G4 40X HandE stain architecture of liver showing prominent hepatocytes separated by dilated sinusoids

### Table 1: Effect of ethanolic extracts *Terminalia arjuna* on lipid profile

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>ANOVA</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG mg/dl</td>
<td>104.5±13.8</td>
<td>134.3±25.1</td>
<td>206.5±90.7*</td>
<td>129±32</td>
<td></td>
<td>4.58</td>
<td>0.013</td>
</tr>
<tr>
<td>TC mg/dl</td>
<td>130.6±13.6</td>
<td>122.8±16</td>
<td>123.1±17.5</td>
<td>185±17*</td>
<td></td>
<td>20.48</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL mg/dl</td>
<td>30.3±1.8</td>
<td>30±1.9</td>
<td>41.8±14 abc</td>
<td>36.8±2.5</td>
<td></td>
<td>3.64</td>
<td>0.03</td>
</tr>
<tr>
<td>LDL mg/dl</td>
<td>80.6±12.5</td>
<td>64.7±18.5</td>
<td>33±15.8abc</td>
<td>122.3±14abc</td>
<td></td>
<td>34.7</td>
<td>0.000</td>
</tr>
<tr>
<td>VLDL mg/dl</td>
<td>22.7±4.2</td>
<td>28±7.1</td>
<td>40±18</td>
<td>25.8±6.4</td>
<td></td>
<td>3.0</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD. ANOVA followed by Post Hoc Tukey’s multiple comparison test. Superscript a, b, c express significant difference between groups. A depicts a comparison with group 1, b depicts a comparison with group 2, c depicts a comparison with group 3. (* ≤0.05). Group 1 is normal control rats, Group II is isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is a hyperlipidemic+Ethanolic extract of *Terminalia Arjuna*.

### Table 2: Effect of ethanolic extracts *Terminalia arjuna* on markers of liver function

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>ANOVA</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Bilirubin mg/</td>
<td>0.6±0.1</td>
<td>0.6±0.1</td>
<td>0.7±0.1</td>
<td>0.8±0.1</td>
<td></td>
<td>2.11</td>
<td>0.13</td>
</tr>
<tr>
<td>Bilirubin mg/ %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGOT U/l</td>
<td>76.6±6.5</td>
<td>51.3±8 a</td>
<td>70±28 b</td>
<td>50±8.1 b</td>
<td></td>
<td>3.38</td>
<td>0.000*</td>
</tr>
<tr>
<td>SGPT U/l</td>
<td>59.5±19</td>
<td>47.1±5.6 a</td>
<td>64.5±12 b</td>
<td>46±3X b</td>
<td></td>
<td>3.03</td>
<td>0.000*</td>
</tr>
<tr>
<td>Serum Protein gm/dl</td>
<td>5.7±0.2</td>
<td>6±0.3</td>
<td>5.7±0.2</td>
<td>5.6±0.2</td>
<td></td>
<td>1.38</td>
<td>0.16</td>
</tr>
<tr>
<td>Serum ALP U/l</td>
<td>2.9±0.1</td>
<td>3.4±0.3 a</td>
<td>3±0.1 b</td>
<td>2.8±0.1 b</td>
<td></td>
<td>6.99</td>
<td>0.002*</td>
</tr>
<tr>
<td>Serum Alb gm/dl</td>
<td>1±0.1</td>
<td>1.3±0.2 a</td>
<td>1±0.1</td>
<td>0.98±0.04 b</td>
<td></td>
<td>5.21</td>
<td>0.008*</td>
</tr>
<tr>
<td>Serum A/G gm/dl</td>
<td>153±16</td>
<td>174±26</td>
<td>187±50</td>
<td>161±18</td>
<td></td>
<td>1.4</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD. ANOVA followed by Post Hoc Tukey’s multiple comparison test. Superscript a, b, c express significant difference between groups. A depicts a comparison with group 1, b depicts a comparison with group 2, c depicts a comparison with group 3. (* ≤0.05). The group 1 is normal control rats, Group II is Isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is a hyperlipidemic+Ethanolic extract of *Terminalia Arjuna*.
We observed significant higher levels of SGPT and SGOT in group 3 compared to group 2 (\(P<0.05\)). Similarly, we observed significant changes for SGPT and SGOT in group 2 and group 4.

There was significantly higher values of Serum albumin and the Serum A/G ratio in group 2 compared to group 1 (\(P<0.05\)). Group 4 has shown significant lesser values of Serum albumin and the Serum A/G ratio compared to group 2 (\(P<0.05\)).

We also observed no significant differences for serum bilirubin, serum protein and serum ALP among all groups.

We observed no significant differences for % body weight gain among all groups at 21st day.

Similarly, we observed significantly decreased in % body weight in group 4 compared to group 1, 2 and 3 (\(P<0.05\)).

<table>
<thead>
<tr>
<th>% Body weight gain</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 21st day</td>
<td>20.6±1.2</td>
<td>22.4±1.7</td>
<td>20.9±2</td>
<td>21.5±1.9</td>
<td>1.27</td>
</tr>
<tr>
<td>At 42nd day</td>
<td>17.5±2</td>
<td>18.6±1.3</td>
<td>19.1±2</td>
<td>14.4±1.3(a^{bc})</td>
<td>8.68</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD. ANOVA followed by Post Hoc Tukey’s multiple comparison test. Superscript a, b, c express significant difference between groups. A depicts a comparison with group 1, b depicts a comparison with group 2, c depicts a comparison with group 3 (\(P \leq 0.05\)). Group 1 is normal control rats, Group II is isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is a hyperlipidemic+Ethanolic extract of Terminalia arjuna.

<table>
<thead>
<tr>
<th>Oregano somatic index</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepato-Somatic Index</td>
<td>0.03±0.003</td>
<td>0.026±0.002(a)</td>
<td>0.02±0.003(a)</td>
<td>0.02±0.002 (a)</td>
<td>14.307</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD. ANOVA followed by Post Hoc Tukey’s multiple comparison test. Superscript a, b, c express significant difference between groups. A depicts a comparison with group 1, b depicts a comparison with group 2, c depicts a comparison with group 3 (\(P \leq 0.05\)). Group 1 is normal control rats, Group II is isocaloric diet fed rats, Group III is hyperlipidemic diet fed rats, Group IV is a hyperlipidemic+Ethanolic extract of Terminalia arjuna.

We observed the significant decrease in the hepatic-somatic index in group 2, 3 compared to group 1. Also significant difference was seen in between group 3 and group 4 (\(P<0.005\)).

**Effect of Terminalia Arjuna (Ethanolic Extract) on liver histopathology**

Fig. 1 and 2, G1 (Group 1) 10X and 40X HandE stain histopathology section of liver shown normal hepatic architecture compressed of hepatic lobules formed by the central vein and the cords of hepatocytes with indistinct sinusoidal dilatation in fig. 3,4,7 and 8. G2 and G4 (Group 2 and 4) 10X and 40X HandE stain shown prominent sinusoidal dilatation.

Whereas G3 (Group 3) 10X and 40X HandE stain histopathology shown lobular architecture of the liver with enlarged hepatocytes containing microvesicular and macrovesicular fatty changes with sinusoidal congestion.

G4 (Group 4) Bark extract of Terminalia arjuna (500 mg/Kg body weight) treated rats histopathology section of liver shown normal hepatic architecture

**DISCUSSION**

In the present study Ethanolic extract of Terminalia arjuna bark was tested for their hepatoprotective activity in albino rats fed with hyperlipidemic diet. The degree of protection was assessed using markers of liver function like serum albumin, serum globulin, serum A/G, SGPT, SGOT and serum ALP along with histopathological study.

The peculiar sign of hepatic damage is leakage of cellular enzymes into the serum. The SGPT, SGOT and ALP are important cellular enzymes [12].

When there is hepatopathy, these enzymes leak into the blood stream from the cytoplasm with an extent of liver damage. AST, ALT and bilirubin levels are commonly measured as an indication of hepatocellular integrity. ALT is frequently used as the biochemical parameter to assess hepatic injury [Anitha] [13]. In the present study, increased levels of serum SGPT, SGOT and ALP in hyperlipidemic rats showed the damage of liver tissue. The decreased level of serum SGOT, SGPT and ALP in hyperlipidemic rats treated with *Terminalia arjuna* showed repair of hepatic cells by restoring cell permeability. Similar findings were observed in Hardik Soni et al. study [9].

In the present study, we observed a nonsignificant increase in bilirubin level in hyperlipidemic rats treated with *Terminalia arjuna* compared to hyperlipidemic rats. In contrast, P Doorika et al. reported significant decrease in bilirubin levels in rats treated with *Terminalia arjuna* [4].

The liver plays an important role in the synthesis of protein like Albumin [15].

Akansha P S, et al. reported decreasing level of albumin, total protein level and damage to the normal architecture of the liver. Similar findings were observed in our study [5].

We observed marked structural alteration, i.e. microvesicular and macrovesicular fatty changes in histopathology of liver of rats fed with high fat diet. Hyperlipidemic rats treated with ethanolic extract of *Terminalia arjuna* is shown normal architecture of liver histology. Ragavan, B., et al. reported in their study, high fat diet induced rat treated with *Terminalia arjuna* bark extract shown to partially reverse the damage (Fatty changes) [16].

**CONCLUSION**

The present findings demonstrated the hepatoprotective effect of *Terminalia arjuna* bark extracts on hyperlipidemic rat models. This plant can be used as hepatoprotecting due to the presence of various bioactive compounds such as phenolics, flavonoids, tannins etc. To explore the precise mechanism of action of specific biological active principles, further processing of the ethanolic fraction of *Terminalia arjuna* is required.

**ACKNOWLEDGEMENT**

I sincerely thank to Dr Kusal K Das Prof. Dept of Physiology. Shri B. M. Patil Medical College, Bijapur, for his valuable suggestions in writing the manuscript.

**CONFLICT OF INTERESTS**

Declare None
REFERENCES


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

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2Principal Sridevi Institute of Medical Science and Research Centre Tumkur  
3Prof Dept of Pathology, Shri B M Patil Medical College Hospital & Research Centre, BLDE University, Bijapur.

**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 Ethanolic 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 Emblica Officinalis regarded as “One of the best rejuvenating herbs”. The fruits of Emblica Officinalis are used as dietary and medicinal purposes by Indian system of medicine. It is commonly known as Amla or Indian gooseberry. The major principles of Emblica Officinalis are hydrolyzable tannins (embilica A & B), Gallic acid flavonides, flavones and ascorbic acid. The Emblica Officinalis exerts various biological functions such as antioxidant, anti-atherosclerosis, antidiabetic, hypolipidemic, gastroprotective and cytoprotective. Along with these functions Emblica Officinalis also produce beneficial effects on liver functions As well as alleviate hyperlipidemia and metabolic syndrome. 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. The hepatoprotective actions of Emblica Officinalis noticed to be mediated by its free radical scavenging, antioxidant and modulation of lipid metabolism. In the present study we tried to prove the effect of Emblica 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. Atherosclerosis is a disease that involves the interplay of several factors like oxidation of lipoproteins, formation of atherosclerotic plaques. Amla like statins acta to inhibit HMGCoA reductase activity and ellagic acid acts to inhibit cholesterol biosyn thesis to cholesterol biosynthesis. Hence the present study investigated the therapeutic efficacy of Emblica Officinalis extract on pathophysiology of liver of hyperlipidemic albino wister rats.

MATERIALS AND METHODS

Materials

Fresh, mature, healthy and good quality fruits of Emblica 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 Emblica 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 22-240°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 sacrification 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 Emblica Officinalis (EEO) (21 days Isocaloric diet + 21 days with EEO) and Group V Fed with high fat diet and EEO Ethanolic extract of Emblica 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 µm thickness, deparaffinized, and rehydrated using standard techniques. The extent of Hyperlipidimic diet-induced necrosis and steatosis was evaluated by assessing morphological changes in liver sections stained with hematoxylin and eosin (H&E), using standard techniques.

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

**STATISTICAL ANALYSIS**

Values are expressed as 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.

### RESULTS

**Table 1**

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

<table>
<thead>
<tr>
<th>% weight gain</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 21st Day</td>
<td>21±1.7</td>
<td>14.7±4.8a</td>
<td>17.5±4.5</td>
<td>21.6±1.2b</td>
<td>15.6±2.2d</td>
<td>5.331 0.003</td>
</tr>
<tr>
<td>At 42nd Day</td>
<td>15.6±0.7</td>
<td>14.7±2.4</td>
<td>17.1±2.4</td>
<td>8.6±2.6abc</td>
<td>8.8±3.4abc</td>
<td>17.6 0.00</td>
</tr>
</tbody>
</table>

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). GroupI 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 21st 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 42nd 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.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>ANOVA</th>
<th>F Value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of Liver</td>
<td>7.73±0.17</td>
<td>8.87±0.95</td>
<td>6.72±0.36</td>
<td>7.67±0.22</td>
<td>6.68±0.83</td>
<td>2.246</td>
<td>0.093</td>
<td></td>
</tr>
<tr>
<td>Organosomatic Index</td>
<td>0.03±0.00</td>
<td>0.03±0.00</td>
<td>0.02±0.00</td>
<td>0.03±0.00</td>
<td>0.02±0.00</td>
<td>1.416</td>
<td>0.258</td>
<td></td>
</tr>
</tbody>
</table>

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). GroupI 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
**Effect of Ethanolic extract of Embilica Officinalis on Hematological parameters in different groups of albino wistar rats.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>ANOVA</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb%</td>
<td>12.8±1.1</td>
<td>12.9±1.2</td>
<td>12.8±0.5</td>
<td>13.3±0.14</td>
<td>12±0.8</td>
<td>1.682</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>7783±2806</td>
<td>7266±2084</td>
<td>5633±1723</td>
<td>7383±2918</td>
<td>6566±1538</td>
<td>0.82</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>7.4±0.9</td>
<td>7.3±0.8</td>
<td>7.3±0.8</td>
<td>8±0.2</td>
<td>6.8±0.3</td>
<td>2.667</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Platelet</td>
<td>8.1±2.6</td>
<td>6.4±2.2</td>
<td>7.6±1.2</td>
<td>9.7±0.3</td>
<td>8.2±2.7</td>
<td>2.041</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>PCV</td>
<td>44.6±5.4</td>
<td>43.6±4.8</td>
<td>42.1±2.3</td>
<td>47.9±1.9</td>
<td>39.8±2.5</td>
<td>3.895</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>MCHC</td>
<td>29.9±1.3</td>
<td>29.6±0.6</td>
<td>30.5±0.8</td>
<td>31±1.7</td>
<td>30.3±1.7</td>
<td>4.286</td>
<td>0.009</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>ANOVA</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG mg/dl</td>
<td>160.1±20.8</td>
<td>140.3±35.9</td>
<td>200.3±93.2</td>
<td>110±23.5</td>
<td>129±32.2</td>
<td>3.80</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>TCmg/dl</td>
<td>130.6±13.6</td>
<td>122.8±16.1</td>
<td>123.1±17.5</td>
<td>131.5±15.8</td>
<td>185±17.1</td>
<td>15.8</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>HDLmg/dl</td>
<td>30.3±1.86</td>
<td>30.0±1.89</td>
<td>41.8±14**</td>
<td>29.6±1.86</td>
<td>36.8±2.5</td>
<td>4.08</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>LDLmg/dl</td>
<td>80.6±12.5</td>
<td>64.7±18.5</td>
<td>33±15.8*</td>
<td>79.8±12.8</td>
<td>122.3±14.1</td>
<td>27.94</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>VLDLmg/dl</td>
<td>22.7±4.2</td>
<td>28±7.1</td>
<td>44±18.8*</td>
<td>22±4.7</td>
<td>25±6.7</td>
<td>3.319</td>
<td>0.026</td>
<td></td>
</tr>
</tbody>
</table>

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). GroupI 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 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*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>ANOVA F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum bilirubin mg%</td>
<td>0.83±0.1</td>
<td>0.6±0.01</td>
<td>0.78±0.1</td>
<td>0.8±0.1</td>
<td>0.68±0.19</td>
<td>2.55</td>
<td>0.06</td>
</tr>
<tr>
<td>SGOT U/L</td>
<td>51.6±8.98</td>
<td>73.6±8.5</td>
<td>70.1±28.9</td>
<td>51.3±8.1</td>
<td>18.6±5.04</td>
<td>13.5</td>
<td>0.00</td>
</tr>
<tr>
<td>SGPT U/L</td>
<td>48.1±4.83</td>
<td>59.5±19.7</td>
<td>64.5±12.5</td>
<td>47.16±5.67</td>
<td>22.83±4.26</td>
<td>12.52</td>
<td>0.00</td>
</tr>
<tr>
<td>Serum protein gm/dl</td>
<td>5.68±0.26</td>
<td>5.71±0.25</td>
<td>5.7±0.25</td>
<td>5.6±0.24</td>
<td>6.01±0.38</td>
<td>1.58</td>
<td>0.21</td>
</tr>
<tr>
<td>Serum albumin gm/dl</td>
<td>2.9±0.16</td>
<td>2.9±0.1</td>
<td>3±0.19</td>
<td>2.86±0.13</td>
<td>3.4±0.34</td>
<td>6.44</td>
<td>0.001</td>
</tr>
<tr>
<td>A/G ratio gm/dl</td>
<td>0.9±0.08</td>
<td>1±0.10</td>
<td>1±0.11</td>
<td>0.98±0.04</td>
<td>1.30±0.24</td>
<td>6.42</td>
<td>0.001</td>
</tr>
<tr>
<td>Alkaline phos U/L</td>
<td>16.3±1.7</td>
<td>15.3±1.6</td>
<td>18.7±5</td>
<td>16.1±1.8</td>
<td>17.2±2.6</td>
<td>1.27</td>
<td>0.30</td>
</tr>
</tbody>
</table>

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 Hepatocytes separated by dilated sinusoids in 10X*

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

Figure 9
*Group V A. Architecture of liver showing Hepatocytes separated by dilated sinusoids in 10X.*
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. And the hyperlipidemic rats treated with Emblica Oficinalis showed normal body weight, lipid profile as well as normal liver histology. We observed decrease in % body weight gain in rats treated with Emblica Oficinalis compared to Group I at 21st day. Also, there was a significant decrease in % body weight gain in rats treated with Emblica Oficinalis compared to hyperlipidemic rats and control at 42nd day. There was no significant difference observed between hyperlipidemic rats treated with Emblica Oficinalis and rats treated with only Emblica Oficinalis at 42nd day, this could be due to excessive breakdown of tissue proteins and catabolism of fats and proteins. 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 Emblica Oficinalis. In addition haemogram showed improved levels of Hb, RBC and other cells. 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 ($p < 0.01$). 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. 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. 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. In our study, histopathological

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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. 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. 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 Hyperlipidimic 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 Hyperlipidimetic 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. 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. 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. Emblica Officinalis contains many liver tonic which can be used against acute viral hepatitis and other liver disorders. 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.

ACKNOWLEDGEMENT

I sincerely thank to Dr Kusal K Das Prof. Dept of Physiology. Shri B M Patil Medical College, Bijapur, for his valuable suggestions in writing manuscript.

REFERENCES


ORIGINAL ARTICLE

Effect of Ethanolic Extract of *Embilica officinalis* on Histopathology of Kidney and on Biochemical Parameters in Hyperlipidemic Albino Rats

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Abstract:
Background: It has been reported that hyperlipidemia plays a central role in the development of atherosclerosis and oxidative stress. *Embilica officinalis* also known as Amla or Indian Gooseberry acts as antihyperlipidemic and antioxidant. Its active ingredients contains tannins, gallic acid and flavonoids. Aim & Objectives: It was aimed to evaluate the effect of ethanolic extract of *Embilica officinalis* on histopathology of kidney and on biochemical parameters in hyperlipidemic albino Wistar rats.

Material and Methods: Extraction of dried fruits of *Embilica officinalis* was done by Soxhlet apparatus using 99% ethanol at 60°C for 24 hours and also phytochemical analysis was done. Group I served as normal control. Group II was fed with isocaloric diet. Group III was fed with hyperlipidemic diet. Group IV was fed with isocaloric diet for 21 days + *Embilica officinalis* for 21 days. Group V was fed with hyperlipidemic diet for 21 days + *Embilica officinalis* for 21 days. The dose of ethanolic extract of *Embilica officinalis* was taken as 100mg/kg body weight daily.

Results: Percent body weight gain, kidney weight and nephro-somatic index significantly improved in hyperlipidemic rats treated with *Embilica officinalis*. There was a significant improvement in serum electrolyte and kidney markers. It was found that there were focal glomerular lesions with thickening of glomerulus in the kidneys of rats on hyperlipidemic diet and normal renal histology of rats on hyperlipidemic diet treated with *Embilica officinalis*.

Conclusion: It can be concluded that *Embilica officinalis* may be a good, natural therapeutic agent against hyperlipidemic diet induced oxidative damage and nephrotoxicity.

Keywords: *Embilica officinalis*, Hyperlipidemic diet, Histopathology of Kidney, Kidney markers, Nephrotoxicity, Serum electrolyte

Introduction:
Medicinal plants are nature's gift for human beings to boost a disease free healthy life. Various medicinal plants are present in a class of herbal preparations of the Indian medicine system [1]. Among many of these herbal drugs *Embilica officinalis* is one of the precious herbal drug, commonly known as Indian gooseberry or Amla which belongs to the family of Euphorbiacae. Amla is very rich in nutrition and can be an important dietary source of Vitamin C, minerals and amino acids etc [2]. The plant also contains phenolic compounds such as tannins, saponins, phyllembelin and embilicanin. The fruit of Amla shows antioxidant, antidiabetic, hypolipidemic, antibacterial and hepatoprotective properties [3]. Along with these functions *Embilica officinalis* also may produce beneficial effects on kidney functions. Kidney contributes major role in electrolyte balance and Blood Pressure (BP) regulation. BP is regulated by renal handling of substances like Na⁺, Cl⁻ and HCO₃⁻. It happens
under the control of renin angiotensin mechanism. In this way it maintains homeostasis of the body [4]. In the present study we tried to find out the effect of Embilica officinalis on histopathology of kidney in hyperlipidemic rats. We have given a hyperlipidemic diet to induce hyperlipidemia in albino Wistar rats to develop an animal model expressing changes in kidneys and kidney markers (serum creatinine and blood urea). High fat diets lead to atherosclerosis. It is a condition that involves the interplay of several factors like oxidation of lipoproteins and atherosclerotic plaques formation [5]. This pathogenesis could induce renal vasoconstriction followed by hypoxic condition in the kidney which promotes further ischemic renal injury. The hypoxic tissue produces reactive oxygen species (ROS) more than antioxidant present in the renal tissue. Amla as an antioxidant contains gallic acid which is a multiple hydroxyl group compound. It donates its proton to break the chain reaction of free radicals acting as an inhibitor to lipid peroxidation [6]. It was aimed to explore the nephroprotective effect of ethanolic extract of Embilica officinalis on renal dysfunction and pathological destructions in a hyperlipidemic rat model.

Material and Methods

Materials:
Healthy and good quality fresh, mature, Embilica officinalis (Amla) fruits were procured from the market, in the months of November–December 2012, and were identified and authenticated by the Department of Botany K.C.P. Science College, Bijapur. Extraction process was conducted in the Department of Pharmacology, BLDEA college of Pharmacy, Bijapur.

Extraction:
300gms of the powder of dried fruits of Embilica officinalis (Amla) was extracted with 99% ethanol using Soxhlet apparatus at a temperature 60°C for 24 hours. The solvent was evaporated under vacuum which gave semisolid mass with respect to the dried powder.

Study design:

Experimental Animals:
Albino Wistar rats weighing 180 to 250gms were obtained from animal house of Shri B M Patil Medical college Hospital and Research Centre, Bijapur. All animals were acclimatized for 7 days to the laboratory conditions at 22-24°C maintaining a 12- hour 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 and Research Centre, Bijapur. All care of animals was taken as per the guidelines of ICMR on animal research (2006) during the experiment as well as at the time of sacrifice. This study was undertaken in the Department of Anatomy. The newborn and old and diseased rats were excluded.

Preparation of Isocaloric Diet:
For 1kg of diet, 180gm of casein, 620gm of carbohydrate, 200gm of fat and 1% of multi vitamin and 2% NaCl were taken [7].

Preparation of Hyperlipidemic Diet:
For 1kg of diet, 180gm of casein, 520gm of carbohydrate, 300gm of fat and 1% of multi vitamin and 2% NaCl were taken [8].

Experimental Protocol
All the rats were divided into following five groups with 6 rats in each group.
Group I served as normal control fed with water and food ad libitum, Group II was fed with isocaloric diet for 42 days, Group-III was fed with high fat diet for 42 days, Group IV was fed with isocaloric diet for 21 days and ethanolic extract of Embilica officinalis (EEO) for 21 days each and Group V was fed with hyperlipidimic diet for 21 days and EEO for 21 days each. 100mg/kg body weight of EEO was given daily. [9]
Sample collection

Every alternate week (six rats) one group of animals were sacrificed by cervical dislocation at the end of the last dose with an overnight fast. Blood was collected in normal tubes for the separation of serum, by doing retro-orbital puncture, before sacrificing the animals.

Tissue collection for histopathology:

After proper dissection of animal kidneys were isolated immediately and fixed in 10% neutral buffered formalin solution for 24 hours [10]. The fixed tissues were processed routinely and then embedded in paraffin, sectioned to 3–5 µm thickness, de-paraffinized, and rehydrated using standard techniques. The extent of hyperlipidemic (high fat diet) induced necrosis was evaluated by assessing morphological changes in kidney sections stained with Hematoxylin and eosin (HandE), using standard techniques.

Gravimetry:

Estimation of Body Weight and Renal-somatic Index of Albino Wister rats:

Procedure 1:
The total body weight of each rat was recorded on the first day (beginning of experiment), Group I was fed with water and food ad libitum, Group III and V were fed with hyperlipidemic diet and Group II and IV were fed with iso-caloric diet for 21 days and the weight was measured. The same diet was continued from 21st to 42nd day. In addition Group IV and V were treated with EEO.

Procedure 2:
After 42nd day again weight was measured and after overnight fast rats were sacrificed. With proper dissection right side kidney was collected and weight was measured to the nearest of 0.1 mg on a digital weighing machine. Further the renal-somatic index was calculated by the formula of kidney weight/total body weight.

Estimation of Biochemical Parameters (Renal Markers and Serum Electrolytes):

Blood Urea, Serum Creatinine, Serum Na' (Sodium), K’(Potassium), Ca’’ (Calcium) and Cl' were analyzed by Meril Diagnostic Kit Method.

Statistical Analysis:

Values were expressed as Mean ± SD. To determine the significance of inter group differences, one way ANOVA followed by 'Post Hoc t tests' were used. P ≤ 0.05 was considered statistically significant.

Results:

Gravimetry

At 21st day: It was observed that there was a significant increase in percent body weight gain in Group III as compared to Group I (p< 0.05). Group IV showed a significant decrease in % body weight gain as compared to Group III (p<0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>ANOVA</th>
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<tr>
<td></td>
<td>F Value</td>
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</tr>
<tr>
<td>On 21st day</td>
<td>14.2 ± 4.7</td>
<td>18.5 ± 4.1</td>
<td>21 ±1.0\a</td>
<td>15±3.8\a</td>
<td>19.8±1.7</td>
<td>5.2</td>
</tr>
<tr>
<td>On 42nd day</td>
<td>14.5 ± 2</td>
<td>16.8 ± 1.4</td>
<td>8.9±3\b</td>
<td>8.9±3\b</td>
<td>14.6±2.1\c,d</td>
<td>12.2</td>
</tr>
</tbody>
</table>

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 V (*P value is ≤ 0.05). Group I -normal control rats, Group II - Isocaloric diet fed rats, Group III- hyperlipidemic diet fed rats, Group IV- EEO fed rats, Group V-hyperlipidemic + EEO fed rats
At 42nd day: It was observed that there was a significant decrease in percent body weight gain in Group III and IV as compared to Group I and II (p<0.05). Group V showed a significant increase in percent body weight gain as compared to Group III and IV (p<0.05).

It was observed that there was significant decrease in body weight of Group II compared to Group I (p<0.05). There was significant increase in body weight of Group III compared to Group II (p<0.05). Kidney weights of Group II and Group III were lower compared to group I. Group IV which showed significant increase in kidney weight compared to Group II and Group III. Group V showed a significant relation with Groups I, II, III and IV (p<0.05). There was a significant decrease in renal somatic index of Group II and Group III compared to Group I. Group V showed significant relation of renal somatic index to Group II and III (p<0.05).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (gm)</th>
<th>Group II (gm)</th>
<th>Group III (gm)</th>
<th>Group IV (gm)</th>
<th>Group V (gm)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>253 ± 11.4</td>
<td>228 ± 19.3</td>
<td>254 ± 9.6</td>
<td>236 ± 6.2</td>
<td>234 ± 10.2</td>
<td>6.24</td>
</tr>
<tr>
<td>Kidney weight (gm)</td>
<td>2.6 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.6 ± 0.09</td>
<td>2.5 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>223.1</td>
</tr>
<tr>
<td>Renal somatic Index</td>
<td>0.01 ± 0.0</td>
<td>0.003 ± 0.0</td>
<td>0.002 ± 0.00</td>
<td>0.01 ± 0.0</td>
<td>0.009 ± 0.0</td>
<td>229.3</td>
</tr>
</tbody>
</table>

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 V (* P value is ≤ 0.05). Group I - normal control rats, Group II - Isocaloric diet fed rats, Group III - hyperlipidemic diet fed rats, Group IV - EEO fed rats, Group V - hyperlipidemic + EEO fed rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Urea (mg%)</td>
<td>31±4.1</td>
<td>28±6.0</td>
<td>30±6.2</td>
<td>31±5.2</td>
<td>27±7.0</td>
<td>0.7</td>
</tr>
<tr>
<td>S. Creatinine (mg%)</td>
<td>0.9±0.1</td>
<td>0.8±0.2</td>
<td>0.7±0.3</td>
<td>0.9±0.1</td>
<td>0.8±0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Na+ (mEq/L)</td>
<td>139±2.0</td>
<td>143±5.0</td>
<td>140±1.5</td>
<td>139±1.8</td>
<td>141±4.9</td>
<td>2.89</td>
</tr>
<tr>
<td>K+ (mEq/L)</td>
<td>4.9±0.7</td>
<td>6.4±0.6</td>
<td>4.6±1.0</td>
<td>4.8±0.7</td>
<td>4.7±0.6b</td>
<td>4.03</td>
</tr>
<tr>
<td>Ca++ (mg/dl)</td>
<td>8.6±0.08</td>
<td>9.9±0.5</td>
<td>8.7±0.1</td>
<td>8.6±0.1</td>
<td>8.7±0.2b</td>
<td>13.2</td>
</tr>
<tr>
<td>Cl- (mEq/L)</td>
<td>98±0.9</td>
<td>97±0.9</td>
<td>97±1.3</td>
<td>97±1.2</td>
<td>98±1.2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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 V (* P value is ≤ 0.05). Group I - normal control rats, Group II - Isocaloric diet fed rats, Group III - hyperlipidemic diet fed rats, Group IV - EEO fed rats, Group V - hyperlipidemic + EEO fed rats
Biochemical Parameters:
No significant differences for Blood Urea, Serum Creatinine and Serum Cl⁻ among all Groups was observed. It is observed that the values for Na⁺, K⁺, Ca²⁺ were all significantly higher for Group II. All the other groups had similar values.

Histopathology of Kidney:
Photomicrography of H and E stained kidney of Group III hyperlipidemic (high fat diet) rats showed focal glomerular lesions including thickening of the glomerulus and normal renal tubules, whereas Group I normal control, Group II isocaloric diet, Group IV isocaloric diet with EEO and Group V hyperlipidemic diet with EEO showed normal microscopic architecture of kidney.
Fig. 4: Histopathology of H and E Stained 100X Kidneys of Group III Hyperlipidemic (High Fat Diet) Rats showing Focal Glomerular Lesions Including Thickening of the Glomerulus and Normal Renal (DCT) Tubules

Fig. 5: Histopathology of H and E Stained 100X Kidney of Group III Hyperlipidemic (High Fat Diet) Rats showing Glomerular Thickening

Fig. 6: Rat Kidney of Group IV Showing Normal Architecture of Kidney and No Histopathological Changes (HandE 40X)

Fig. 7: H and E stained (40 X) Kidney of Group V Hyperlipidemic (High fat diet) Rats Treated with EEO showed Histopathological Changes
Discussion:
The study was aimed to demonstrate the nephroprotective effect of EEO to prevent the development of renal dysfunction and alteration of histopathology of kidney which are assessed by biochemical and renal markers in a hyperlipidemic rat model. Studies on such models significantly add to the knowledge towards enriching in the field of medical research. Hyperlipidemic animal models expressed changes in renal markers, biochemical parameters and histopathology of kidney. As Emblica officinalis being a potent antioxidant it exerts free radical scavenging activity and shows preventive role against fat induced renal toxicity [11].

It was observed no histopathologic change and no alteration of renal markers were found in rat kidneys of Group V and they also retained normal body weight. It indicates that EEO has exerted nephroprotective effect in rat kidney even when it was given hyperlipidemic diet. EEO has revealed to attenuate hyperlipidemia in Group V. EEO may be helpful to restore renal functioning due to its presence of gallic acid and tannins [6]. An increase in percent body weight gain in rats treated with EEO (Group IV) was observed compared to control rats (Group I) at 21st day (p<0.05). Also there was a significant increase in percent body weight gain in rats treated with EEO (Group IV) compared to hyperlipidemic rats treated with EEO (Group V) at 42nd day (p<0.05). There was a significant decrease in percent body weight gain in rats treated with Embilica officinalis (Group IV) compared to control rats at 42nd day (p<0.05).

Adis et al found that serum creatinine and bilirubin levels increased significantly in contrast media group compared to control group. Pretreatment with EEO demonstrated its nephroprotective effect by attenuating the severity of renal pathological damage and improving renal functioning [6]. In contrast we found non significant decrease in serum creatinine and blood urea in Group V compared to Group I. Eteng et al showed a slight non significant increase in serum (Na+) level for Group I (100mg/kg body weight) Wistar rats [12].

We observed that Na and K levels were significantly lower in Group III, IV and V compared to Group II (p<0.05). Also there was significant increase in levels of K in group II compared to Group I (p<0.05). Ca level was significantly higher in Group II compared to Group I (p<0.05). Increased serum levels of triglycerides and cholesterol causes to lipid accumulation in different organs and tissues like arterial wall, liver, kidney and muscles [13]. In our study we observed the effect of (high fat diet) hyperlipidemia on the kidneys of albino Wistar rats. The hyperlipidemia caused minimum percent of lipid accumulation in kidney tissue. Histopathology of renal corpuscles exhibited normal appearance in Groups I and II (Fig.1, Fig.2) whereas Group III showed the prominent increase in the glomerular capillaries (Fig. 3, 4 and 5). Microscopic study of high fat diet i.e. Group III rat kidney showed focal glomerular lesions including thickening of glomerular changes and completely decreased space (space for urine) between glomerulus and Bowmen's capsule. Epithelial cell lining of renal tubules were hemorrhagic and appeared dilated compared to normal histology of kidney. Fig. 6 and 7 both show normal histopathology, Fig. 4 and 5 show changes due to hyperlipidemia and Fig. 7 shows normal histopathology after high fat diet and treatment with EEO.

Experimental studies have shown that hyperlipidemic diet may be associated with increased oxidative stress in animals [14].

In our study we tried to assess the influences of Emblica officinalis on renal functions and histopathology in hyperlipidemic albino Wistar rats. From the results, Embilica officinalis is useful in regulating hyperlipidemia and
histopathology of kidneys in albino Wistar rats fed with hyperlipidemic diet.

**Conclusion:**
In conclusion, we describe hereby distinctive renal glomerular ultrastructural injury associated with hyperlipidemia and markedly reduced alteration in renal glomerular histopathology when treated with fruit extract of *Embilica officinalis*. We would like to state that fruit extract of *Embilica officinalis* plays a protective role against hyperlipidemic diet induced oxidative damage and nephrotoxicity. The mechanistic pathways by which *Embilica officinalis* acts as a protective therapeutic against that particular toxin is not fully clear except its antioxidant property. Further investigations are necessary to find the active functional group(s) of the compound(s) of *Embilica officinalis*. However extensive clinical studies are required to establish the efficacy and safety of *Embilica officinalis* for treating multiple pathologies associated with hyperlipidemia.

**Acknowledgement:**
I sincerely thank to Dr Kusal K Das, Prof. Dept of Physiology, Shri B M Patil Medical College, Bijapur for his valuable suggestions during conducting present study as well as in writing manuscript.

**References**

Paper presentations in Conference and Seminars

ORAL PRESENTATION

1. 14th Karnataka state Anatomy Conference at Shri B M Patil College, Hospital & Research Centre, BLDE University Bijapur 8-9th September 2012.
   Topic: Effect of Terminalia Arjuna and Emblica Officinalis Extract on Cardiovascular System in Albino Wister Rats

POSTER PRESENTATIONS

1. Application to Nanotechnology in Health Care June 13, 2012 at BLDEA’s College of Pharmacy, Bijapur
   Topic: Effect of Terminalia Arjuna and Emblica Officinalis Extract on Cardiovascular System in Albino Wister Rats

   Topic: Effect of Terminalia Arjuna and Emblica Officinalis Extract on Cardiovascular System in Albino Wister Rats

3. 14th Karnataka state Anatomy Conference at Shri B M Patil College, Hospital & Research Centre, BLDE University Bijapur 8-9th September 2012.
   Topic: Effect of Terminalia Arjuna and Emblica Officinalis Extract on Cardiovascular System in Albino Wister Rats
From
Dr. R.S. Wali
Chairman,
Institutional Animal Ethics Committee (IAEC),
Prof. & HOD, Dept. of Pharmacology,
BLDEU's Shri. B.M. Patil Medical College,
BIJAPUR.

To,
Dr. Bheemshetty S Patil,
Lecturer, Dept. of Anatomy,
BLDEU's Shri. B.M. Patil Medical College,
BIJAPUR.

ETHICAL CLEARANCE CERTIFICATE

The Institutional Animal Ethics Committee (IAEC) of this College met on 31.05.2011 at 10.30am to scrutinize the Research Project submitted by faculty member of this College.

After scrutiny the following research project has been accorded ethical clearance

Title: "Effect of Terminalia Arjuna and Emblica Officinalis extract on cardiovascular system in albino wister rats"

Principal Investigator: Dr. Bheemshetty S Patil, Lecturer, Dept. of Anatomy.

[Signature]
Dr. R. S. Wali
Chairman, (IAEC)
Prof. & HOD,
Dept. of Pharmacology
BLDEU's Shri. B. M. Patil Medical College,
BIJAPUR.

[Signature]
Professor & HOD,
Dept. of Pharmacology
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BIJAPUR.
EFFECT OF TERMINALIA ARJUNA AND EMBLICA OFFICINALIS EXTRACT ON CARDIOVASCULAR SYSTEM IN ALBINO WISTER RATS