

# Histological Evaluation of Aqueous Extracts of *Phyllanthus Emblica* Fruit And *Terminalia Arjuna* Bark on Body Weight, Hepatic and Renal Structures In High Fat Diet Induced Albino Wistar Rats

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## ABSTRACT

**Back ground:** High fat diet is one of the leading causative factors for high cholesterol levels. Hyperlipidemia plays a major role in the development of atherosclerosis and oxidative stress. **Aim& Objective:** The current study was taken to assess the high fat diet changes in the body weight, hepatic and renal tissue treated with the various doses of aqueous extracts of *Phyllanthus emblica* fruit and *Terminalia arjuna* bark. **Materials and Methods:** Group I served as normal control. Group II was fed with high fat diet. Group III was fed with high fat diet and aqueous extract of *Phyllanthus emblica* fruit -200 mg/kg body wt. Group IV was fed with high fat diet and aqueous extract of *Phyllanthus emblica* fruit -400 mg/kg body wt. Group V was fed with high fat diet and aqueous extract of *Terminalia arjuna* bark -400mg/kg body wt. Group VI was fed with hyperlipidemic diet + Atorvastatin standard drug. **Results:** Histopathological analysis using hematoxylin and eosin stain for liver, renal glomerulus and tubules showed therapeutic effect with treatment of *Phyllanthus emblica* fruit extract 400mg/kg b.w and *Terminalia arjuna* bark extract 400mg/kg b.w. **Conclusion:** we observed the high fat diet induced structural and histological changes in the liver and renal tissue which was improved with *Phyllanthus emblica* fruit extract 400mg/kg compared with *Terminalia arjuna* bark.

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## INTRODUCTION

The liver is the primary organ which plays an important role in metabolic and excretion and maintains homeostasis of the body. Hyperlipidemia is greatest risk factors for prevalence and severity of coronary heart diseases and liver damage.<sup>1</sup> 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 from toxin effects. The modern system of medicine is no exception. It cures one hand and trigger side effects on the other.<sup>2</sup> There is increasing demand of people towards an Ayurvedic system of medicines which shows reduced adverse effects on health. *T.arjuna* belong to the family combretaceae which is a cardioprotective in nature in ayurveda. Its bark has active principles like glycosides, flavonoids, tannins and minerals. Flavonoids acts like antioxidants, anti-inflammatory and lipid lowering effect where as glycosides are cardio tonic. It also shows hepato-protective effect.<sup>3</sup>

Hyperlipidemia is caused due to genetic factor, high intake of the dairy products, fatty food products with unbalanced diet due to more intake of carbohydrates and fat consumption with lack of physical exercise which leads to the disturbance in the liver metabolism and also in the kidney functioning.<sup>4</sup> The cardiovascular diseases result in the formation of arterial thickening called arteriosclerosis which shows its effect on the liver, kidney and brain.<sup>5</sup> Hyper cholesterol can be lowered by using naturally available sources like *Phyllanthus emblica* commonly known as Indian gooseberry or Amla belongs to the family Euphorbiaceae.<sup>6</sup> The chemical constituents of *Phyllanthus emblica* includes tannins, alkaloids, phenols, flavanoids and also contains high amount of vitamin-c with antioxidant Activity,<sup>7</sup> two hydrolysable tannins Emblicanin-A and B which had antioxidant properties.

There are several allopathic drugs available to treat hyperlipidemia but most of them are causing side effects. This suggests there is a need for the discovery of effective and safe anti-hyperlipidemic agents,

## KEYWORDS:

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Atherosclerosis  
High fat diet,  
*Phyllanthus emblica*,  
Renal glomerulus,  
*Terminalia arjuna*.

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preferably of natural origin.<sup>8</sup> *Phyllanthus emblica* (Amla) is one of the most important medicinal plants in Indian ayurveda, widely used as diuretic, laxative, liver tonic, refrigerant, stomachache, restorative, anti-pyretic and ulcer protective as alone or in combination with other herbal drugs.<sup>9</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. The hepatoprotective actions of *Phyllanthus embilica* noticed to be mediated by its free radical scavenging, antioxidant and modulation of lipid metabolism.

Among vital organs kidney is the first organ showing altered functions due to effect of high fat diet. High fat diet affects positive energy balance and causes ectopic accumulation of fat in intracellular compartments. Renal fat deposition makes deleterious effect on kidney functions and land up in lipotoxicity in renal tissue. Lipotoxicity is the pathological situation where lipid peroxidation occurs and generates reactive oxygen species (ROS). Overproduction of ROS than antioxidant present in tissues cause oxidative stress.<sup>10</sup> In this scenario a promising therapeutic approach for the prevention of adverse effect of high fat diet is recommended. However, several synthetic modern medicines are available which are expensive and have been associated with many unacceptable adverse effects. Hence much focus has been given for safe and effective therapeutics profile of plant based that have very less side effects.

The protective role of *T. arjuna* in the kidneys especially compared to an adverse effects of lipids on other organs like heart, liver and skeletal muscle. With this point of view the present study was designed to assess protective role of aqueous extract of bark of *T. arjuna* against high fat induced nephrotoxicity and oxidative stress.

The Global Registry of Acute Coronary Events (GRACE) aimed to achieve pronounced reductions in low density lipoprotein levels. Higher dose statin use could be considered as a contributing factor to renal damage in older people, especially in females. The role of dyslipidemia in promoting kidney damage has been shown in experimental models when

administered to rats with kidney disease caused by unilateral nephrectomy, the cholesterol and fat rich diet augmented the glomerular lesions in the remaining kidney.<sup>11</sup> Lipid deposition can directly damage the glomerular basement membrane and also stimulate mesangial cell (contractile cells that constitute the central stalk of the glomerulus) activation and proliferation, a process similar to smooth muscle cell proliferation in the evolution of atherosclerotic plaque. *Phyllanthus emblica* (syn. *Emblica officinalis*), commonly known as Indian gooseberry or 'amla' is a major rejuvenating component of herbal drugs used in ayurvedic systems of medicine.<sup>12</sup> In this view the study was taken to check the renal tissue, liver damage with the high fat diet and also to retain the normal structure of the kidney with the effective drug like aqueous extract of *Phyllanthus emblica fruit* and *Terminalia arjuna* bark where in the present conditions most of the people opting for natural or herbal drugs for which this study was selected.<sup>13</sup>

## MATERIALS AND METHODS

### Drugs used in this study

1. Aqueous extracts of *Phyllanthus emblica* fruit -200 & 400 mg/kg body wt
2. Aqueous extract of *Terminalia arjuna* bark -400 mg/kg body wt
3. High Fat Diet: Edible vegetable oil and Vanaspathi procured from local market.
4. Atorvastatin standard drug 20mg.
5. Histological tools - Alcohol solutions, xylene, paraffin wax, tissue blocks microtome, microscope .
6. Stains used - Hematoxylin and Eosin stain.

**Animals:** The male albino wistar rats of 180-250g were taken from the animal house of pharmacology department (MIMS) Maharajah's Institute of Medical Sciences, Nellimarla, Vizianagaram, India.

### Plant Extract

Aqueous extracts of *Phyllanthus emblica* fruit (AEPEF) and *Terminalia arjuna* bark (AETAB) were obtained from Bhagavathi herbals, Gujarat, India.



**Fig. 1:** *Phyllanthus emblica* fruit and bark of *Terminalia arjuna*

**Qualitative estimation of Phytoconstituents:** The collected extracts were analyzed to explore chemical profile using standard phytochemical tests qualitatively

### Selection of Animals

The experimental protocol was approved by the Institutional Animal Ethics Committee of M.I.M. S Medical College, Nellimarla, Vizianagaram. The study was carried out in healthy male albino wistar rats weighing 180-250g. Animals were maintained under a standard animal diet of rat pellets for a period of 8 weeks. The animals were weighed, recorded, numbered, and randomly divided into 6 groups of 5 animals each for a period of 8 weeks according to CPCSEA (Committee for the purpose of control and supervision of experiments on animals) for laboratory animal facilities. All care was taken for animals during experiment as well as at the time of sacrifice as per the guidelines of CPCSEA No. 753/03/C/CPCSEA.

**Induction of hyperlipidemia:** High fat diet (HFD) was prepared by mixing vanaspati and palm oil in the ratio of 3:2 for a period of 8 weeks for all the groups except for normal control group which were fed with the standard rat pellets and all the animal groups were provided with sufficient water.

### Acute Toxicity Study

The acute toxicity of aqueous extracts of *Phyllanthus emblica* fruit and *Terminalia arjuna* bark was determined. Rats were fasted overnight and randomly divided into four groups with five rats per each group. Extract doses were given as per OECD guidelines as 1000 and 2000 mg/kg body wt and were administered separately to the rats of each group using bulbed steel needle. All rats were then allowed free access to food and water and observed over a period of 48 hr for signs of acute toxicity. The number of deaths within this period was recorded.

**Experimental Design:** The animals were divided into six groups, five animals in each group.

- **Group I:** Normal- albino wistar rats
- **Group II:** High Fat Diet - control
- **Group III:** High fat diet + aqueous extract of *Phyllanthus emblica* fruit (AEPEF) - 200mg/kg body wt.
- **Group IV:** High fat diet + aqueous extract of *Phyllanthus emblica* fruit (AEPEF) - 400mg/kg body wt
- **Group V:** High fat diet + aqueous extract of *Terminalia arjuna* bark (AETAB) - 400mg/kg body wt.
- **Group VI:** High fat diet + Atorvastatin (standard drug)

All the animals used for the study were kept under observation for the food intake. The drugs were administered to the animals for 8 weeks daily except for the normal control group through intragastric feeding tube at the end of the 8<sup>th</sup> week all the rats were sacrificed.

### Sample and Tissue Collection

All the six 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, after mild ether anesthesia the dissection was approached by vertical incision through the cervical region followed to pelvic region. The necessary tissue samples for the experiment like renal, liver samples were collected and kept in 10% formalin solution which was then followed for the tissue processing and staining.

### Gravimetry

Estimate the body weight of Albino wistar rats.

### Procedure

The body weight of each rat was recorded on the initial day and last day of the experiment with single pan electronic balance. The body weight of all rats was recorded at the beginning of the experiment (day 1), treatment with an aqueous extract of *Phyllanthus emblica* fruit and *Terminalia arjuna* bark (28<sup>th</sup> day) and on the day of sacrifice (56<sup>th</sup> day). Liver weight was



**Fig. 2:** Albino wistar rats



measured in a single pan balance (Digital weighing machine) and Hepato-somatic index was calculated by the formula (liver weight/total body weight) X100

### Biochemical Analysis

In biochemical analysis, we estimated liver and kidney marker levels and serum electrolytes

#### Estimation of Liver Function Tests

Serum Bilirubin, Serum Alkaline Phosphatase (ALP) , Serum Glutamic Oxalocetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) were analysed by Meril diagnostic Kit method.

#### Estimation of Biochemical Parameters (renal markers and Serum Electrolytes)

Blood urea, Serum creatinine, Serum Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Cl<sup>-</sup> were analyzed by CJASN diagnostic kit method.

#### Assessment of Oxidative Stress

Oxidative stress marker like malondialdehyde (MDA) which is an end product of lipid peroxidation was estimated in serum. Serum MDA estimation was done by thio-barbituric acid reactive substance (TBARS) assay using spectrophotometer of wavelength 535 nm. The absorbance of was read at 535 nm using spectrophotometer.

### 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

### Preliminary and qualitative analysis of Phytoconstituents

The qualitative phytochemical analysis of aqueous extracts of *Phyllanthus emblica* fruit (AEPEF) and *Terminalia arjuna* bark (AETAB) was shown in the (table1).

### Gravimetry

On 28<sup>th</sup> day it was observed that there was a significant increase in percent body weight gain in Group-II, III, IV, V and VI as compared to Group-I (p <0.05) but there was insignificant body weight gain between all the experimental groups compared to Group-II. On 56<sup>th</sup> day it was observed that there was a significant decrease in percent body weight gain in Group-III, IV, V and VI as compared to both Group I (p <0.05) (Table-2)

### Estimation of Liver and Kidney Marker Levels and serum Electrolytes

Blood urea, creatinine, Na<sup>+</sup> and Cl<sup>-</sup> levels were showed insignificant difference in Groups III,IV,VI compared to Group II (High fat diet rats) but K<sup>+</sup> and Ca<sup>2+</sup> levels showed significant difference in all treated groups (Group III,IV, V& VI groups) when compared to Group II (High fat diet rats).(Table-3 & 4). In our study we observed the effect of (high fat diet) hyperlipidemia on the liver and kidneys of albino wistar rats which causes minimum percent of lipid accumulation in kidney tissue.

### Oxidative Stress

Table 6 depicts significant increased levels of serum MDA and decreased levels of Vitamin C in rats fed with high fat diet indicate increased lipid peroxidation and oxidative stress. We observed significant decrease in MDA levels in rats supplemented with high fat fed diet and aqueous extract of *Phyllanthus emblica* fruit 400mg/kg body wt (Group IV) compared to Group III & V). There is a significant increase in vitamin C in Group 4 compared to aqueous extract of *Phyllanthus emblica* fruit 200mg/kg body wt (Group III) and aqueous extract of *Terminalia arjuna* bark 400mg/kg body wt (Group V). (Table 6)

**Table 1:** Qualitative phytochemical analysis of aqueous extracts of *Phyllanthus emblica* fruit and *Terminalia arjuna* bark

S.No	Phytochemicals	AEPEF	AETAB
1	Alkaloids	-	+
2	Tannins	+	+
3	Proteins	-	-
4	Saponins	+	+
5	Coumarins	-	-
6	Flavanoids	-	+
7	Phenols	+	+
8	Carbohydrates	+	+
9	Glycosides	+	+
10	Sterols	-	+

+ indicates Present - indicates Absent  
AEPEF: Aqueous Extract of *Phyllanthus emblica* fruit

**Table 2:** Effect of AEPEF and AETAB on Percent of body weight gain

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Anova	
							F value	P value
On 28 days	15.67 ± 1.97	21.67 ± 2.34#	20.17 ± 2.64#	19.50 ± 2.66#	19.86 ± 2.84#	16.67 ± 2.07 #	6.75	0.0007
On 56 days	16.3 ± 31.51	22.33±2.46#	15.67±1.51*	14.33 ± 1.51*	15.34± 1.52*	12.67 ± 2.73**	5.35	0.002

Values are expressed as mean ±SD. ANOVA followed by Post Hoc Tukey's multiple comparison test. . #p<0.01 compared to normal control group, \*\*p<0.01,\*p<0.05 Compared to Highfat diet rats. Group I -normal control rats, Group II - hyperlipidemic diet fed rats, Group III- High fat diet + AEPEF -200mg/kg body wt, Group IV - High fat diet + AEPEF -400mg/kg body wt, Group V - High fat diet + AETAB -400 mg/kg/body weight, Group VI - High fat diet + Atorvastatin (standard drug)

**Table 3:** Effect of aqueous extract of Phyllanthus emblica fruit and Terminalia arjuna bark extract on body weight change, liver and kidney weight, renal, hepato- somatic index

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Anova	
							F value	P value
Body weight (gm)	239.6±10.73	291.40±4.21#	271.8±10.96#,**	263.1 ± 5.04#,**	269.4 ± 4.77	260.2 ± 4.65#,**	17.92	0.0001
Kidney weight (gm)	2.84±0.05	0.94±0.21#	2.36±0.22#,**	2.52 ±0.11#,**	2.44 ± 0.22	2.78 ± 0.11#,**	88.69	0.0001
Renal somatic Index	0.011±0.00	0.003±0.00#	0.006±0.00#,**	0.008 ± 0.00#,**	0.008 ± 0.00	0.009 ± 0.00#,**	49.56	0.0001
Liver weight (gm)	7.54±0.26	6.6±0.40	6.84±0.53	7.12±0.63	6.76 ± 0.57	7.46±0.55	2.91	0.0339(ns)
Hepato-somatic Index	0.03±0.00	0.028±0.00	0.29±0.00	0.03±0.00	0.03±0.00	0.03±0.00	1.52	0.246

Values are expressed as mean ±SD. ANOVA followed by Post Hoc Tukey’s multiple comparison test. . #p<0.01 compared to normal control group, \*\*p<0.01,\*p<0.05 Compared to Highfat diet rats. Group I -normal control rats, Group II - hyperlipidemic diet fed rats, Group III- High fat diet + AEPEF -200mg/kg body wt, Group IV - High fat diet + AEPEF -400mg/kg body wt, Group V - High fat diet + AETAB -400 mg/kg/body weight, Group VI - High fat diet + Atorvastatin (standard drug)

**Table 4:** Effect of Aqueous Extract of Phyllanthus emblica fruit, Terminalia arjuna bark on Kidney Markers and Serum Electrolytes

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Anova	
							F value	P value
Blood urea (mg %)	34 ± 1.58	34.8± 1.09	33.2 ± 3.34 NS	32.60± 2.40 NS	32.8 ± 3.03	33.8 ± 2.28 NS	0.583	0.712
Serum creatinine (mg %)	0.9 ± 0.07	0.68 ± 0.23	0.82± 0.15NS	0.8 ± 0.14 NS	0.78 ± 0.13	0.92 ± 0.08 NS	2.04	.126
Na+ (mEq/L)	136± 2.0	141.6 ± 6.54	139 ± 4.35 NS	138.2± 0.44 NS	138.8± 4.15	135.2 ± 1.78 NS	1.831	0.144
K+ (mEq/L)	4.6 ± 0.14	6.72 ± 0.16	5.04 ± 0.43**	4.88± 0.41**	4.96± 0.33	4.3 ± 0.68 **	21.83	0.00001
Ca2+ (mEq/L)	8.48 ± 0.22	9.6 ± 0.2	8.7 ± 0.38**	8.6± 0.2**	8.72±0.37	8.28 ± 0.54 **	9.39	0.000
Cl- (mEq/L)	96.4 ± 2.07	98.8± 0.44	98 ± 1.22 NS	97 ± 1.41 NS	97.8± 1.10	96.4± 2.07 NS	2.06	0.105

Values are expressed as mean ±SD. ANOVA followed by Post Hoc Tukey’s multiple comparison test. (NS-Not significant Compared to High fat diet rats) \*\*p<0.01, Compared to High fat diet rats. Group I -normal control rats, Group II - hyperlipidemic diet fed rats, Group III- High fat diet + AEPEF -200mg/kg body wt, Group IV - High fat diet + AEPEF -400mg/kg body wt, Group V - High fat diet + AETAB -400 mg/kg/body weight Group VI - High fat diet + Atorvastatin (standard drug)

**Table 5:** Effect of aqueous extract of Phyllanthus emblica fruit and T.arjuna bark on Liver enzyme markers parameters in high fat diet induced albino wistar rats

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Anova	
							F value	P value
Serum Bilirubin	0.86 ± 0.05	0.62 ± 0.18	0.72 ± 0.11NS	0.78 ± 0.11 NS	0.74 ± 0.09 NS	0.82 ± 0.08 NS	2.881	0.335
Serum ALP	53.4 ± 2.30	86.2 ± 3.90	67.8 ± 4.44*	64.8 ± 15.01*	66.4 ± 20.59*	59.2 ± 5.59*	5.141	0.002
Serum SGOT U/L	44.8 ± 4.97	80.4 ± 8.17	55.2 ± 6.30**	52.4 ± 4.83**	53.4 ± 3.71**	50.4 ± 3.91**	25.24	0.0001
Serum SGPT U/L	45.6 ± 2.61	77.6 ± 5.13	58.4 ± 5.55**	54.6 ± 4.98**	56.4 ± 3.29**	52.8 ± 3.63**	30.77	0.0001

Values are expressed as mean ±SD. ANOVA followed by Post Hoc Tukey’s multiple comparison test. (NS-Not significant Compared to High fat diet rats) \*\*p<0.01, Compared to High fat diet rats, \*p<0.05, Compared to High fat diet rats Group I -normal control rats, Group II - hyperlipidemic diet fed rats, Group III- High fat diet + AEPEF -200mg/kg body wt, Group IV - High fat diet + AEPEF -400mg/kg body wt, Group V - High fat diet + AETAB -400 mg/kg/body weight, Group VI - High fat diet + Atorvastatin (standard drug)

**Liver marker enzymes**

There is an insignificant difference in serum bilirubin levels in extracts treated groups (Groups III, IV, V) and standard drug treated group (Group VI).There is a significant decrease in the serum alkaline phosphatase in Group IV compared with Group II (p<0.05). There is a significant decrease in the serum SGOT level in Group IV compared with Group III and V (p<0.01) and significant decrease in serum SGPT levels in Group IV compared with Group II (p<0.01). (Table 5)

**Histopathology analysis**

For histopathological evaluation, paraffin blocks were made for hepatic and renal tissues preserved in formalin. The tissue sections of 5-µm thickness were cut using microtome (Leica RM 2125, Germany). Histopathology of all tissues were stained with Hematoxylin and Eosin (H&E) and studied under light microscope.

**Histopathology of Liver: Hepatic lobules (Fig:3)**

*Group I (Normal)*

Histopathology section of liver shown hepatic architecture with prominent polygonal hepatic lobules containing rounded vesicular nuclei with central vein and cords of hepatocytes are radiating like spokes of wheel from the central vein, fenestrated plates of liver cells, separated from each other by large vascular spaces known as hepatic sinusoids were observed. The peri portal area was also clear and evident. The portal triad with the vessels and duct was clear.

**Group II (High fat diet)**

Histopathology of liver showed disturbed lobular architecture of the liver with enlarged hepatocytes, (ballooning) degeneration of hepatocytes with extensive steatosis with sinusoidal congestion and with evident focal lesions, reduced portal

space. Most of the cells showed cytoplasmic vacuolations. Some of the cells showed multiple vacuoles. There was a cellular infiltration close to the central vein. There was 70% of the lipid accumulation in the high fat diet group compared to normal group. The prominent portal triad was not observed with the bile duct, portal vein, and hepatic artery. The central vein had shown much dilated and enlarged in the high fat diet wistar rats. Both macro-vesicular and micro-vesicular vacuoles were seen, out of which maximum amount of macro vesicular cells made its appearance.

**Group III, IV**

Whereas with the administration of the aqueous extract of *Phyllanthus emblica* fruit and *T.arjuna* bark extract at 400 mg dose along with HFD retained the hepatocytic cytoplasm structure with the intact structural epithelium of the central vein and with normal hepatic sinusoids and also with clear portal triad.

**Group V**

Whereas with the administration of the aqueous extract of *T.arjuna* bark 400 mg dose along with HFD retained the hepatocytic cytoplasm structure with the intact structural epithelium of the central vein and with mild hepatic sinusoids and also with clear portal triad

**Group VI**

Histopathology of liver shown prominent hepatocytes but with mild micro-vesicular fatty - changes and also with dilated central vein.

**Histopathology of kidney: Renal Glomerulus and tubules (Fig:4)**

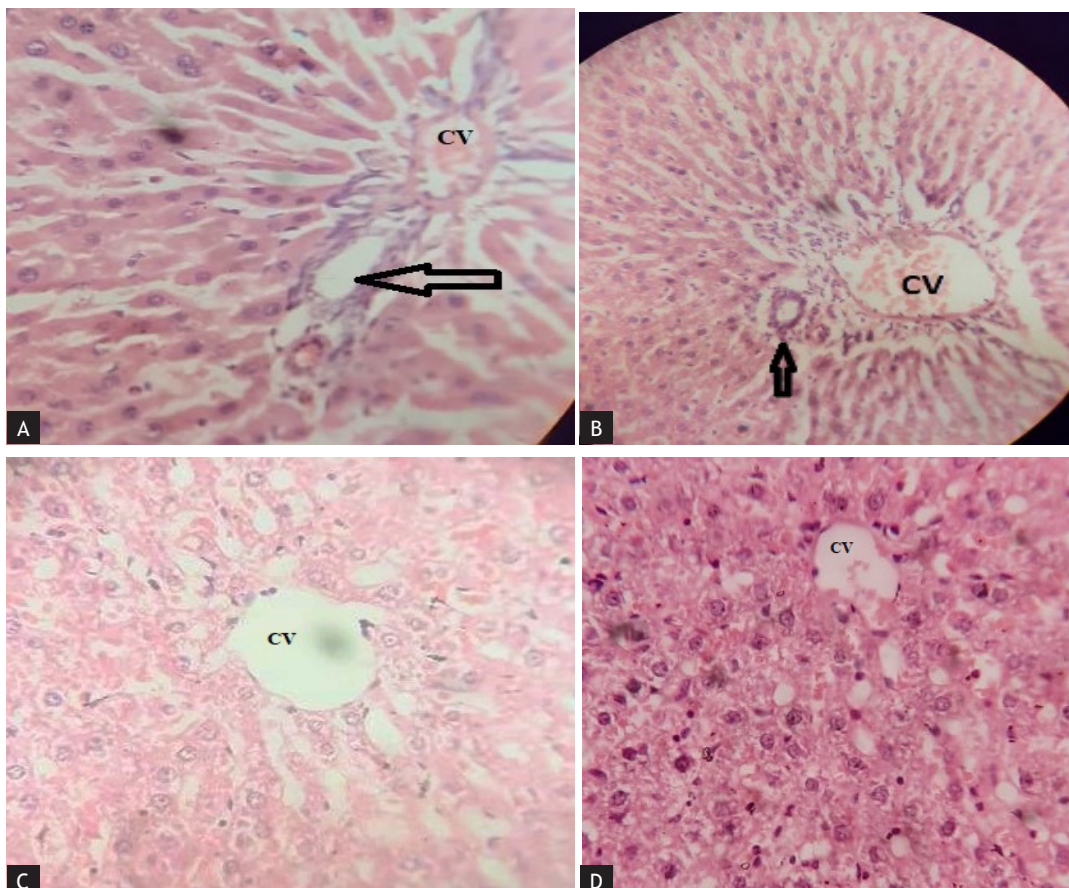
**Group I**

The renal tissue of control group rats showed cortex with clear glomerulus, vessels, tubules with clear cytoplasm, distinct nucleus in the glomerulus which was surrounded by the

**Table 6:** Effect of aqueous extract of Phyllanthus emblica fruit and T.arjuna bark on Lipid peroxidation parameters in high fat diet induced albino wistar rats

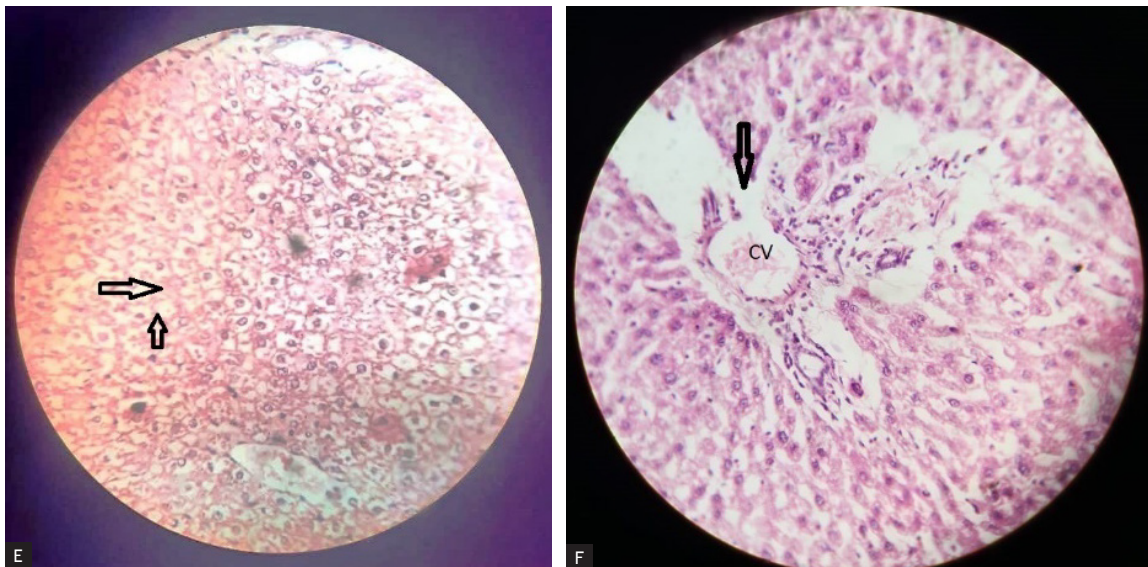
Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Anova	
							F value	P value
MDA	1.076±0.01	4.58±0.16	2.34±0.22**	1.95±0.66**	2.16±0.22**	1.65±0.59**	47.63	0.0001
VITAMIN-C	3.8 ± 0.43	1.62 ±0.18	2.84±0.55**	3.28±0.30**	2.56±0.26**	2.16±0.22**	24.90	0.0001

Values are expressed as mean ±SD. ANOVA followed by Post Hoc Tukey’s multiple comparison test. (NS-Not significant Compared to High fat diet rats) \*\*p<0.01, Compared to High fat diet rats, \*p<0.05, Compared to High fat diet rats Group I -normal control rats, Group II - hyperlipidemic diet fed rats, Group III- High fat diet + AEPEF -200mg/kg body wt, Group IV - High fat diet + AEPEF -400mg/kg body wt, Group V - High fat diet + AETAB -400 mg/kg/body weight, Group VI - High fat diet + Atorvastatin (standard drug)

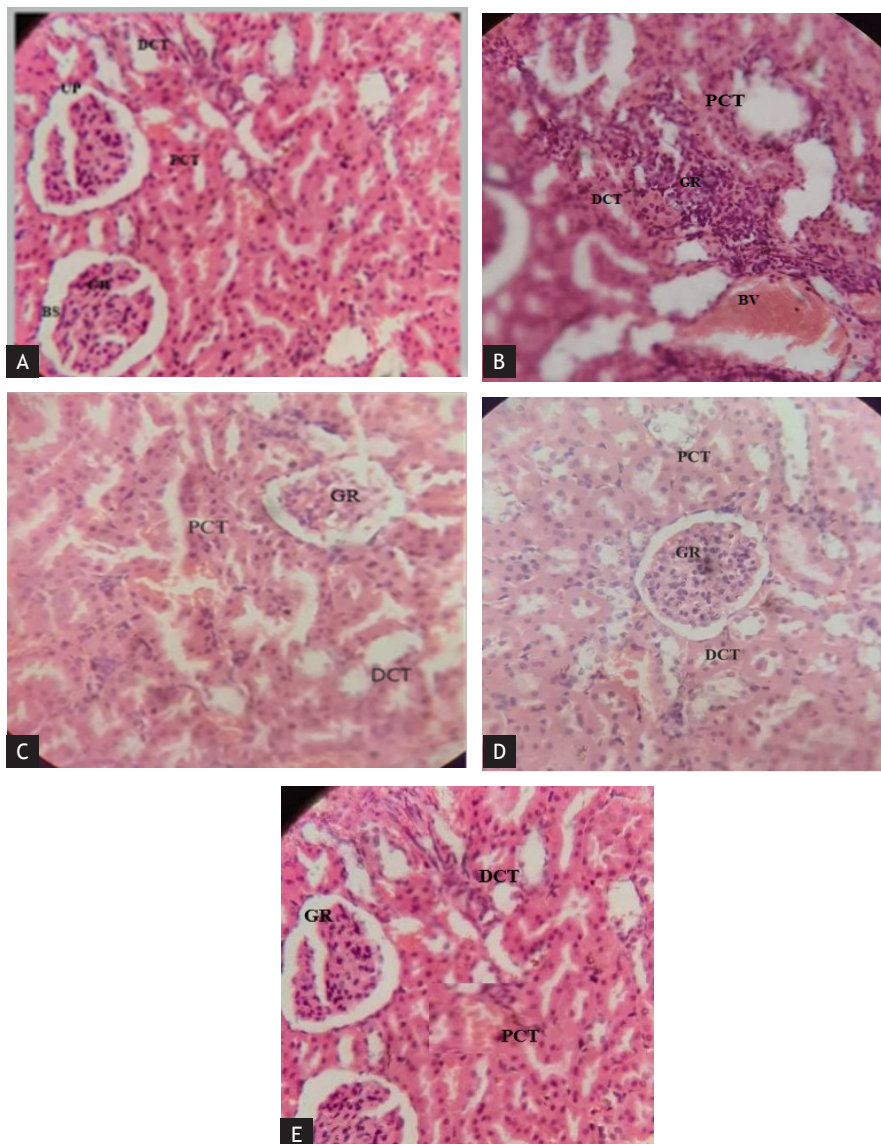


**Fig. 3:** Liver sections indicating hepatic cords with prominent





Black transparent arrows in (E) showing microvesicular and macrovesicular fatty change. Black arrow in (F) showing distortion in central vein epithelium of Group II.(HFD).



**Fig. 4:** Kidney sections stained with Haematoxylin and Eosin (A) Group-I (Control group) (B) Group-II (High Fat diet treated rats) (C) Group-III (High fat diet + aqueous extract of Phyllanthus emblica fruit-400mg/kg body wt. treated rats) (D) Group-IV (High fat diet + aqueous extract of Terminalia arjuna bark -400mg/kg body wt. (E) Group-V (High fat diet + Atorvastatin -Standard drug)

PCT-proximal convoluted tubules, DCT-Distal convoluted tubules ,BV-Blood vessel, GR-Glomerulus.BS-bowman's space

bowman's capsule without any thickening of the cells, and also the structure of proximal and distal convoluted tubules (PCT and DCT) showed little interstitium with cuboidal and brush border epithelium in the tubules.

### Group II

The glomeruli of kidney from High fat diet group showed glomerular capillaries degeneration with dilatation of the renal tubules and congestion of the afferent and efferent arterioles with migrating foam cells in the glomerulus tuft of capillaries. There was segmental necrosis with degeneration of nephron nuclei and in the renal Medulla their tubular cells degeneration.

### Group III, IV

In the Group III and Group IV rats the glomerulus showed squamous epithelium, glomerular tuft of capillaries with decreased thickness of endothelium in the Bowman's capsule.

### Group V

In this group the rats glomerulus showed squamous epithelium, glomerular tuft of capillaries with decreased thickness of endothelium in the Bowman's capsule.

### Group VI

In Group VI there was mild decrease in the thickening in the cellular structure of the Bowman's capsule.

## DISCUSSION

Hyperlipidemia, hypercholesterolemia has become the major problem due to food habits and life style. In the present study hyper cholesterol or high fat diet was induced to the animals with vanaspati and edible oil in the ratio of 3:2<sup>(14)</sup>. This study assessed the body weight changes and the nephroprotectivity and hepatoprotectivity. There was significant decrease in the body weight in the Groups -III, IV, V, VI compared to Group-II whereas there was decrease in percent body weight gain in Groups IV compared to group III, V.

In the high fat diet group rats the liver tissue showed as hepatic lobules with enlarged hepatocytes as micro and macro steatosis with congestion of sinusoids. whereas the hyper-lipidemic rats treated with the aqueous fruit extract of *Phyllanthus emblica* and *T.arjuna* at dose of 400 mg/kg body weight for 28days followed the normal group pattern. This study also showed the similar observations like the study of Bheemsetty etal with the hyperlipidemic rats and isocaloric diet rats of ethanolic extract of *emblica officinalis* and ethanolic extract of *T.arjuna* bark at a dose of 100 mg/ kg body weight with the high fat diet for a period of 42 days<sup>(15)</sup>.

In the previous studies of mice models with high fat diet showed obesity which led to renal deformities with histopathological changes such as dilatation, tubular defects in the kidney structure<sup>(16)</sup>. This study also showed glomerular thickening, tubular changes in the kidney structure. The blood recirculation and filtration in the kidney involves mainly within the proximal convoluted tubules. The raise in the lipid levels lead to atherosclerosis which could also show its adverse effect on the impairment of the other vital organs like kidney.

There are certain herbal drugs for the hyperlipidemic therapy that could bring significant protection against the atherosclerosis and coronary artery disease, out of which for this study the aqueous fruit extract of *Phyllanthus emblica* is considered to identify the changes along with the serum creatinine and protectivity of the renal tissue compared to *Terminalia* bark extract<sup>(17)</sup>. In some of the hyper cholesterol studies it was showed that the oxidative stress is involved during ageing process for renal damage with increased level of serum creatinine but showed marked changes in the levels of serum creatinine after treated with ethyl acetate extract of Amla fruit<sup>(18)</sup>, whereas in the present study the aqueous fruit extract of *Phyllanthus emblica* was given along with the high fat diet to the albino wistar rats which improved the renal structure compared to group II rats with the dose of 400mg /kg body weight (Group IV) where it showed it's protective activity for high fat diet treated for the renal tissue. It also acts as an antioxidant with free radical scavenging activity showing preventive role against fat induced renal toxicity<sup>(19)</sup>.

In our study we tried to assess the influences of *Phyllanthus emblica* aqueous fruit extract and *T.arjuna* on renal morphometry and histopathology in hyperlipidemic albino wistar rats. From the results, aqueous fruit extract of *Phyllanthus emblica* is useful in regulating hyperlipidemia and histopathology of kidneys in albino wistar rats fed with hyperlipidemic diet.

In the present study HFD group showed slight necrosis and cellular swelling in renal tubules however tubular damage was less evident in group VI receiving (HFD+AV). Hyperlipidemia was induced with high fat diet and along with it the treatment drug aqueous bark extract of *T.arjuna* 400mg/kg body wt and fruit extract of *Phyllanthus emblica* was given as 200mg/kg body wt & 400 mg/kg body weight and the improved tubular structure was more with prominent epithelium and lumen in the tubules with *Phyllanthus emblica* than *terminalia arjuna*

In the ethanolic extract of *Emblca officinalis* fed with isocaloric diet and hyperlipidemic group at dose of 100mg/kg body weight showed thickening of the glomerulus only with normal distal convoluted tubules in hyperlipidemic group<sup>(20)</sup>. Where as in the present study there was thickening of the renal tubules also along with glomerulus in the group II. The groups treated with the doses of 400 mg/kg body weight followed the normal group pattern compared to Group II, in Group VI it was less evident than the herbal treated groups. Experimental studies have shown that hyperlipidemic diet may be associated with increased oxidative stress in animals<sup>(21)</sup>.

## CONCLUSION

In conclusion, the microscopic structure of this study showed that there could be changes with the high fat diet intake and structural changes to the liver and kidney which was improved with the natural sources like *Phyllanthus emblica* and *Terminalia arjuna*. Distinctive renal glomerular ultra-structural injury associated with hyperlipidemia and markedly reduced alteration in renal glomerular histopathology when treated with fruit extract of *Phyllanthus emblica* compared to other groups which plays a protective role against hyperlipidemic diet induced oxidative damage and nephrotoxicity. The mechanistic pathways by which *Phyllanthus emblica* acts as



a protective therapeutic against that particular toxin is not known clearly except its antioxidant property. Further investigations are necessary to find the active functional group(s) of the compound(s) of *Phyllanthus emblica* and *Terminalia arjuna*. Extensive clinical studies are required to establish the efficacy and safety of *Phyllanthus emblica* and *Terminalia arjuna* for treating multiple pathologies associated with hyperlipidemia.

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