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# Anti-Hyperlipidemic Activity of *Vateria indica* Linn. Seed Butter in High Fat Diet Induced Hyperlipidemia in Rats

Rajmohan, Suma Venkatesh Mallya<sup>1</sup>, Mohamed Faisal<sup>1\*</sup>, Niranjana Prabhu<sup>1</sup>, Mundugaru Ravi<sup>2</sup>, Ravishankar Basavaiah<sup>3</sup>  
Kottakkal Arya Vaidyasala, Udayanagar, Bangalore 560016. <sup>1</sup>Department of Dravyaguna, SDM College of Ayurveda, Kuthpady, Udupi 574118. <sup>2</sup>Research Officer (Pharmacology), <sup>3</sup>Director, SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi 574118, India.

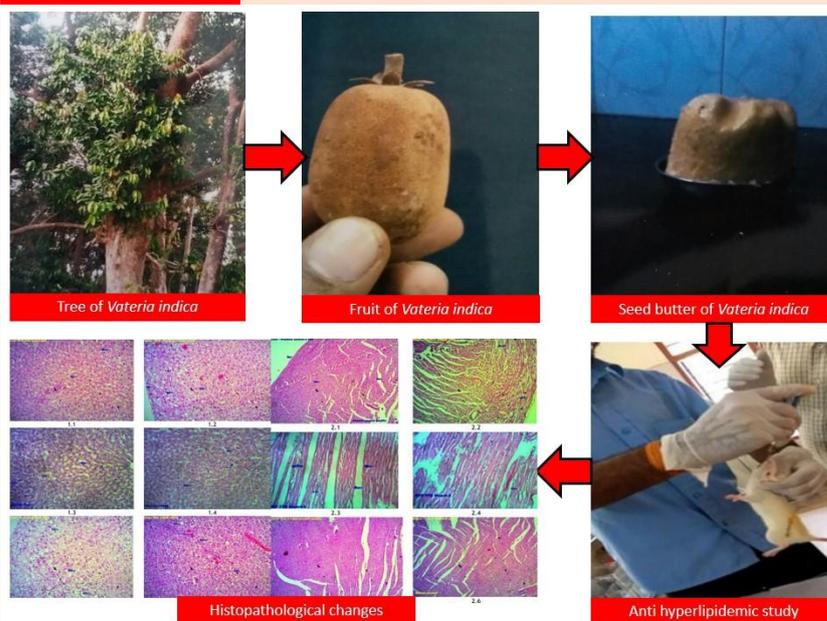
## ABSTRACT

**Introduction:** Use of medicinal herbs in the form of food is a famous Ayurvedic art of life prescribed before traditions. *Vateria indica* Linn. (*Sarja*) seed butter (Indian tallow) forms a source of edible oil in the household of Udupi and Dakshina kannada Districts of Karnataka. Research and addition of therapeutically useful species enables to preserve the local wealth of tradition and also enriches the existing pharmacopeia. Hence an experimental study was planned to evaluate anti hyperlipidemic activity of *V. indica* seed butter in albino rats against high fat diet induced hyperlipidemia in Wistar albino rats. **Methodology:** The 40 animals were divided into five different groups consisting of six rats each. The first normal control group maintained with normal rat diet and water ad libitum. The second positive control group was administered with hyperlipidemic diet (40% cholesterol suspended in hydrogenated vegetable oil, 0.5 ml/100g). The third standard group was administered with Atorvastatin (10mg/kg *po*). The fourth and fifth trial groups were administered with *V. indica* seed butter single and double dose (0.43 & 0.86 ml/kg body weight) respectively. **Results:** The test drug exhibited marginal, moderate increase in HDL cholesterol, moderate decrease in LDL cholesterol, marked reduction in triglycerides with nephroprotective activity. **Conclusion:** *Vateria indica* seed butter produced significant anti-hyperlipidemic activity through normalizing the serum lipid profile. Cytoprotective action has also been revealed by the histopathological examination of liver, heart and kidney tissue.

## KEYWORDS

Atorvastatin, Cholesterol, Histopathology, Hyperlipidemia, Triglyceride.

## PICTORAL ABSTRACT



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## CORRESPONDENCE

Dr Mohammed Faisal  
Assistant Professor  
Dept of Dravyaguna  
SDM College of Ayurveda  
Udupi 574118, India.  
Email: drfaisalsaheb@gmail.com

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## 1. Introduction

Ayurveda the ancient science of life, evolved through the experiential wisdom and selfless dedication of our seers. Healthy body and diseases are nothing but the outcome of Ahara. The bioenergy is supplied by proper and adequate nutrition in the form of its essential constituents like protein, carbohydrates, fats, minerals, vitamins and water. In spite of tremendous development the plants still remain one of the major source of food and medicine. The traditional system of medicine still uses different plant sources as a source of edible oil according to season geographical condition, age, diseases condition etc.

saturated sources of fat. Chemically it is said to be containing 40-45% stearic acid, 10-13% palmitic acid, and 43-48% oleic acid.<sup>[5]</sup>

*Vateria indica* Linn. is a large evergreen tree distributed in Western Ghats upto 1200m along the streams, commonly known as Indian copal tree.<sup>[1]</sup> The fruit kernels form a source of edible fat termed Malabar tallow. The tree flowers between March to April which implies the plant fruits there after.<sup>[2]</sup> Ayurveda identifies this drug as a source of *Sarja*, possessing anti-hyperlipidemic properties.<sup>[3]</sup> Fruits should be collected immediately when falls to avoid germination. Later fruits are decorticated, kernels separated and dried in shade. Collection of these fruits and selling to herbal vendors is the main trade in coastal districts of Karnataka. Traditionally seeds are crushed and boiled in water till the melted fat rises to the surface. Local people use this in confectionary and as an adulterant.<sup>[4]</sup> Tallow is considered as anti-lipidemic than other

Hyperlipidemia refers to elevated levels of lipids and cholesterol in the blood, also identified as dyslipidemia, being primary risk factor of coronary heart disease and atherosclerosis.<sup>[6]</sup> There are many synthetic products found to reduce serum lipids, such as HMG-CoA reductase inhibitors (statins), which are the most effective and best tolerated drugs, bile acid binding resins, nicotinic acid, fibric acid derivatives and cholesterol absorption inhibitors ezetimibe.<sup>[7]</sup> Despite of the availability of number of anti-hyperlipidemic drugs, therapy still deprived of the efficient safer and economical drugs. Herbal medicines are considered to be safer and free from side effects than synthetic drugs. There are number of plants reported to possess anti-hyperlipidemic activity such as *Allium sativum*, *Commiphora mukul*, *Glycine max* and *Nigella sativum*.<sup>[8-10]</sup>

The butter extracted from seeds of *V. indica* Linn. is used as anti-hyperlipidemic drug by folklore practitioners around coastal Karnataka. The data available in unpublished form at folklore medicine research unit of SDM College of Ayurveda, Udupi. As a folklore claim the drug is selected for the study for the first time, aimed to explore the anti-hyperlipidemic activity of butter extracted from the seeds of *V. indica* Linn in high fat diet induced hyperlipidemia in Wistar albino rats.

## 2. Materials and methods

### 2.1 Test drug preparation

*Vateria indica* Linn. fruits were collected from Udupi district of Karnataka, India during the month of June 2013. The plant material were authenticated by Pharmacognosy department at SDM Centre for Research In Ayurveda and Allied Sciences, Udupi and the specimen deposited at SDM centre of research and allied sciences, Udupi for future references (Voucher number 286/13073003-04). The traditional method is used to extract out Piney Tallow from the seed of *V. indica*. Physical impurities were removed from fruits, seed kernels were removed and shade dried. Later these were made into thick paste using grinding machine. Paste was poured into boiling water, in a copper vessel and stirred constantly with a spatula. Heated over low flame until oil globules appear over the surface of water. These fat materials were transferred into another steel vessel and reheated until water content was removed. Then it was collected and stored in an air tight container, used for current study.

### 2.2 Experimental Animals

Wistar strain albino rats of either sex weighing about 200±50g were procured from animal house attached to S.D.M Research Centre for Ayurveda and Allied Sciences, Udupi. The experimental protocol was approved by Institutional Ethical Committee (CPCSEA/IAEC/SDM/DG02). The animals were fed with normal diet, water ad libitum. They were acclimatized in the laboratory condition for one week prior to the experimentation. The housing provided with, controlled lighting of 12:12h light and dark cycle, temperature of 25°C and relative humidity of approximately 50%. The dose of *Vateria indica* Linn. seed butter (VISB) is calculated on the basis of body surface area ratio by referring to the standard table of Paget and Barnes (1964).<sup>[11]</sup> On this basis, the rat dose was found to be 0.43 mg/kg body weight. The test was carried out in two different dose levels 0.43mg/kg considered as therapeutic dose and 0.86mg/kg considered as double therapeutic dose. The test drug was administered orally to animals with the help of oral catheter.

### 2.3 High fat diet preparation

In some section ballooning of hepatocytes was also observed. But in Atorvastatin treated group above changes were found to be

The high fat diet was prepared freshly every day by 40 % cholesterol suspended in hydrogenated vegetable oil (Vipro Vanaspathi). The suspension was administered at a dose of 0.5ml/100g body weight.

### 2.4 Experimental procedure

The rats were divided into five different groups with six in each group. Group I considered as normal control group, administered with normal rat diet, rat pellet procured from Sai Durga Feed, Bangalore and water *ad libitum*. Group II (High fat diet) - administered for 28 consecutive days. Group III (Reference standard) - administered with Atorvastatin 10 mg/kg. Group IV & V administered with VISB at 0.43mg and 0.86mg/kg body weight. All the groups except normal control were administered with high fat diet for 28 consecutive days. On 29<sup>th</sup> day after overnight fasting the animals were weighed and blood was collected to check the serum biochemical parameters like Total lipid profile, urea, creatinine, SGOT, SGPT and Alkaline phosphatase by retro-orbital puncture for biochemical test and all the animals were sacrificed. Liver, heart and kidney were excised out, cleaned, weighed and transferred to 10% formalin solution to tissue fixation prior to histopathological examination.<sup>[11]</sup>

### 2.5 Statistical analysis

The obtained data were expressed as Mean ± SEM and analyzed by one way ANOVA followed by Dunnet's multiple t-test using Graph Pad Prism 3. A p < 0.05 were considered as statistically significant.

## 3. Results and discussion

### 3.1 Effect on high fat diet induced hyperlipidemia

High fat diet significantly increased serum total cholesterol, triglycerides and LDL-C as compared to normal control. Test drug (VISB) and Atorvastatin significantly reduced the elevated serum LDL-C and triglyceride levels and moderately decreased total cholesterol as compared to the high fat diet control with P < 0.05. Atorvastatin significantly increased serum HDL-C whereas test drug at both dose levels moderately increased as compared to high fat diet control (Table1).

### 3.2 Effect on serum biochemical parameters

High fat diet significantly increased serum SGOT, SGPT and ALP levels and decrease in the serum urea level as compared to normal control. The test drug didn't alter the elevated serum biochemical parameters with P < 0.05; whereas Atorvastatin significantly elevated serum SGOT, ALP and urea as compared to high fat diet control (Table 2).

### 3.3 Effect on percentage change in body weight and relative weights of liver, heart and kidney

The cholesterol control group has shown significant decrease in percentage change in the body weight in comparison to normal control group with P < 0.05; whereas the test drug and standard drug administered group has shown non- significant changes in the percentage change in body weight. The high fat diet group has shown significant decrease in the relative heart weight as compared to normal control group (Table 3).

Histopathological examination of Liver tissue has shown marked changes in high fat diet group. It has shown signs of cell depletion, micro and macro changes in the hepatocytes and inflammatory changes in the lobules in the form of cell infiltration and edema.

markedly reduced. Whereas in test drug administered group changes were similar as high fat diet group, but intensity was on the lower side (Figure 1).

Microscopic examination of heart section has shown normal cytoarchitecture in both Atorvastatin and test drug treated groups (Figure 2).

Microscopic examination of Kidney sections from HFD control group showed mild to moderate fatty degenerative changes in four out of six rats. Mild changes were seen in remaining two rats. In reference standard group these changes were minimum. In test group

receiving the TED dose of the test drug the hyperlipidemia induced fatty changes were not reversed to significant extent (Figure 3). Increase in the level of cholesterol and triglycerides usually occur because of the abnormalities in the synthesis, degradation and transport of their lipoprotein particles. Hyperlipidaemic condition is associated with increased risk of atherosclerotic and cardiovascular diseases. It has been reported that increased levels of plasma total cholesterol and LDL are directly related to a greater incidence of coronary heart diseases. The elevation in triglyceride level is associated with the risk of atherosclerosis. The high level HDL-C has been considered as a protective factor for the development of these diseases.<sup>[12]</sup>

**Table 1. Effect of *Vateria indica* Linn. seed butter on lipid profile**

Group	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglycerides (mg/dl)
Normal control	31.6± 6.008	17.86±1.05	14.85±1.90	65±6.83
Cholesterol control	81.4 ± 5.44 <sup>@</sup>	26.16±4.028	28.00 ±6.37 <sup>@</sup>	337.5±38.08 <sup>@</sup>
Atorvastatin + cholesterol	64.4 ± 7.17	42.66±4.73 <sup>#</sup>	14.5±1.258 <sup>#</sup>	146±16.09 <sup>#</sup>
VSB (0.43mg/kg) + cholesterol	76.75 ± 6.32	27.68±4.410	18.43±2.16 <sup>#</sup>	383.5±19.692
VSB (0.86mg/kg) + cholesterol	74.75 ±5.3.092	33.8±2.68	19.83±2.023 <sup>#</sup>	215.33±32.625 <sup>#</sup>

Data in Mean ±SEM, \*P < 0.05; \*\*p<0.01, @-compared with normal control, #-compared with cholesterol control

**Table 2. Effect of *Vateria indica* Linn. seed butter on biochemical factors**

Group	Urea(mg/dl)	Creatinine (mg/dl)	SGOT (IU/L)	SGPT ( U/L)	ALP(mg/dl)
Normal control	34.16± 1.01	0.61±0.17	88.33±5.57	31.16±2.34	359.5±49.93
Cholesterol control	19.83±2.12 <sup>@</sup>	0.66±0.05	159±10.03 <sup>@</sup>	79.5±5.64 <sup>@</sup>	814.4±149.6 <sup>@</sup>
Atorvastatin + cholesterol	32±2.51 <sup>#</sup>	0.67±0.03	175.5±6.90	103±3.58 <sup>#</sup>	1312.4 ±122.8 <sup>#</sup>
VISB (0.43mg/kg) + cholesterol	13.12±1.05	0.53±0.35	164.83±8.39	85.58±9.26	785±116.8
VISB (0.86mg/kg) + cholesterol	15.16±1.44	0.55±0.06	154.5±8.16	87±6.06	959±72.14

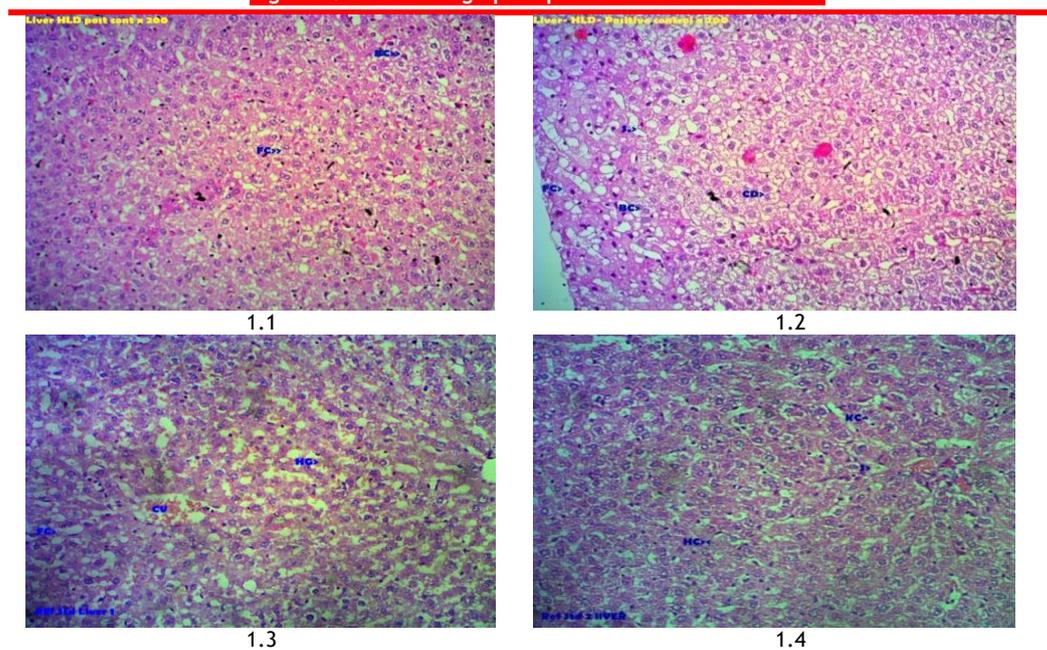
Data in Mean ±SEM, \*P < 0.05; \*\*p<0.01, @-compared with normal control, #-compared with cholesterol control

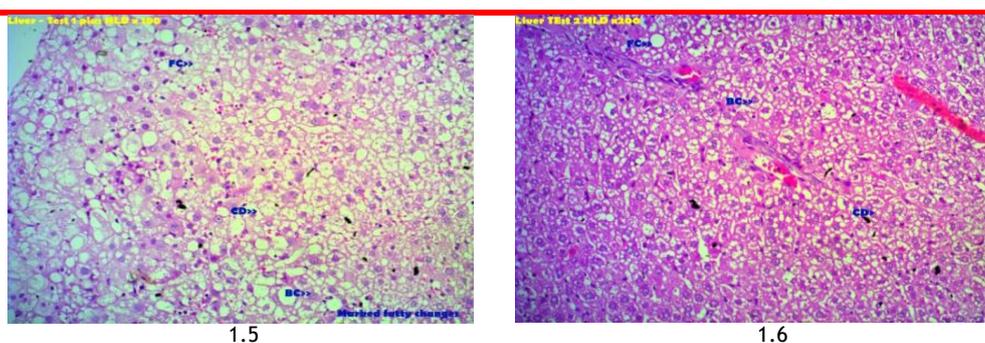
**Table 3. Effect of *Vateria indica* Linn. seed butter on body weight and relative organs weight**

Group	Body weight % change	Relative organ weight		
		Liver	Heart	Kidney
Normal control	6.93±3.69	8.6±0.45	1.81±0.06	0.88±0.02
Cholesterol control	3.49±2.29 <sup>@</sup>	8.12±0.3	1.21±0.04 <sup>@</sup>	0.77±0.44
Atorvastatin + cholesterol	27.16±2.02	8.57±0.68	1.36±0.97	0.61±0.03 <sup>#</sup>
VISB (0.43mg/kg) + cholesterol	7.19 ± 1.8	8.94±0.54	1.57±0.09 <sup>#</sup>	0.84±0.03
VISB (0.86mg/kg) + cholesterol	6.2±1.5	9.31±0.61	1.47±0.97	0.79±0.03

Data in Mean ±SEM, \*P < 0.05, \*\*p<0.01, @-compared with normal control, #- compared with cholesterol control

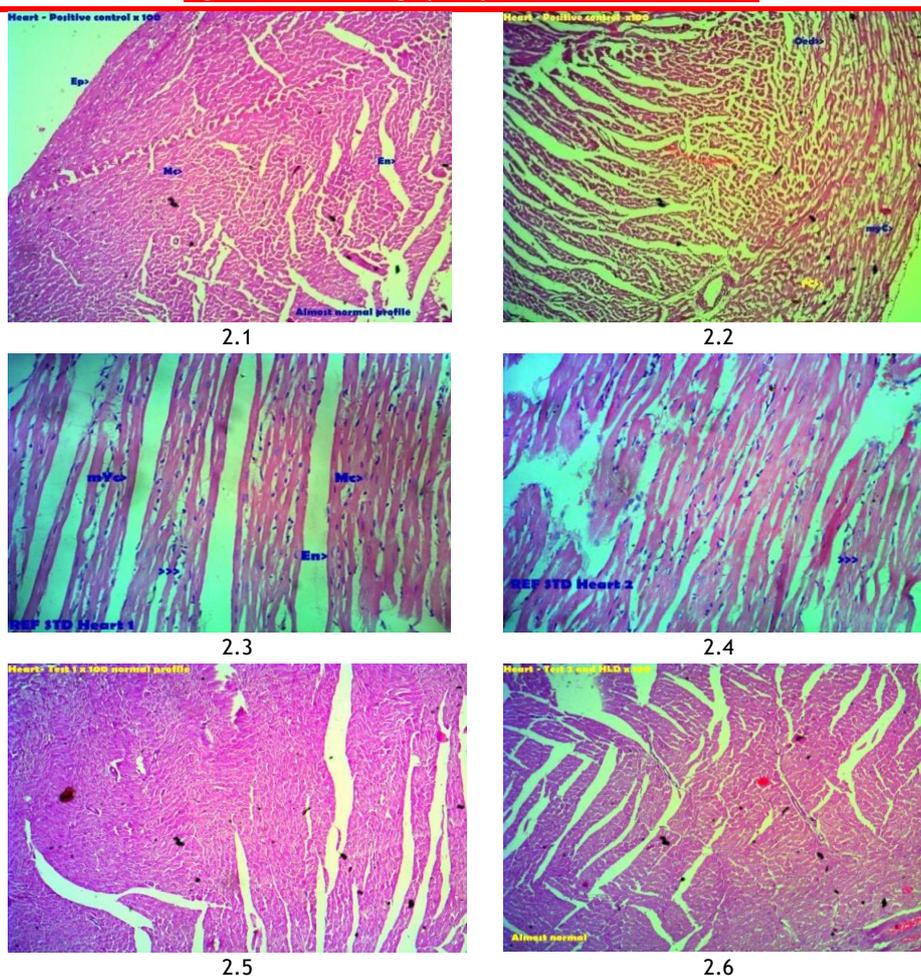
**Figure 1. Photomicrograph representative of heart tissue**





Cholesterol control group (1.1 and 1.2), Reference standard treated with Atorvastatin (1.3 and 1.4), VISB represented in (1.5 and 1.5). FC- Fatty changes, HC: Hepatocytes, CD- Cell degeneration, NC- Normal cyto structure, BC- Ballooning of cells. Cholesterol control group has shown marked changes in Liver cells. They have shown signs of cell depletion, micro and macro changes in the hepatocytes and inflammatory changes in the lobules in the form of cell infiltration and edema. In some section ballooning of hepatocytes was also observed. The Atorvastatin and test drug administered at higher dose level has shown nearly normal cytoarchitecture.

Figure 2. Photomicrograph representative of heart tissue



Cholesterol control group (2.1 and 2.2), Reference standard treated with Atorvastatin (2.3 and 2.4), VISB represented in (2.5 and 2.6). Ep- Epicardium, Ode- Oedema, my C- Myocarditis, Mc - Myocardium, En - Endocardium. Microscopic examination of heart section has shown mild oedematous changes in cholesterol control group; whereas the test drug and Atorvastatin administered group has shown normal cytoarchitecture.

In the present study high fat diet group has shown significant increase in the total cholesterol as compared to the normal control. The Atorvastatin and test drug (VISB) administration has considerably reduced the elevated total cholesterol level. From the earlier studies it has been reported that the Cholesterol acetyl transferase-2 (ACAT-2) is an isoenzyme found in the intestine and liver, where cellular free cholesterol is esterified before triglyceride rich lipoproteins are assembled. In intestine ACAT-2 regulates the absorption of dietary cholesterol.<sup>[13]</sup> Another possible mechanism is inhibition of HMG CoA reductase a rate limiting enzyme in the biosynthesis of cholesterol.<sup>[14]</sup> Thus the test drug might have either inhibition of absorption of dietary cholesterol or has inhibitory action on HMG CoA reductase enzyme.

The LDL particles are arising mainly from the catabolism of LDL, which accounts for the high plasma concentration of LDL. In the hyper triglyceridemia two third of plasma cholesterol is found in the LDL. And the plasma clearance of LDL particle is mediated primarily by LDL receptors located in the liver, which removes more than 75% of LDL from the plasma.<sup>[15]</sup> In the present study the test drug (VISB) at two different dose levels has shown significant reduction in the LDL-C level. It might due to its capacity to induce more number of LDL receptors in the liver and thereby decreasing the plasma LDL- C level.

HDL is considered as protective lipoproteins that decrease the risk of coronary heart disease. This protective effect may result from participation of HDL in reverse cholesterol transport, the process by which the excess cholesterol is acquired from cells and transferred to the liver for excretion.<sup>[16]</sup> In the present study the Atorvastatin has shown significantly increase in the HDL- C level and test drug has showed a moderate elevation. Thus test drug has shown moderate protective activity.

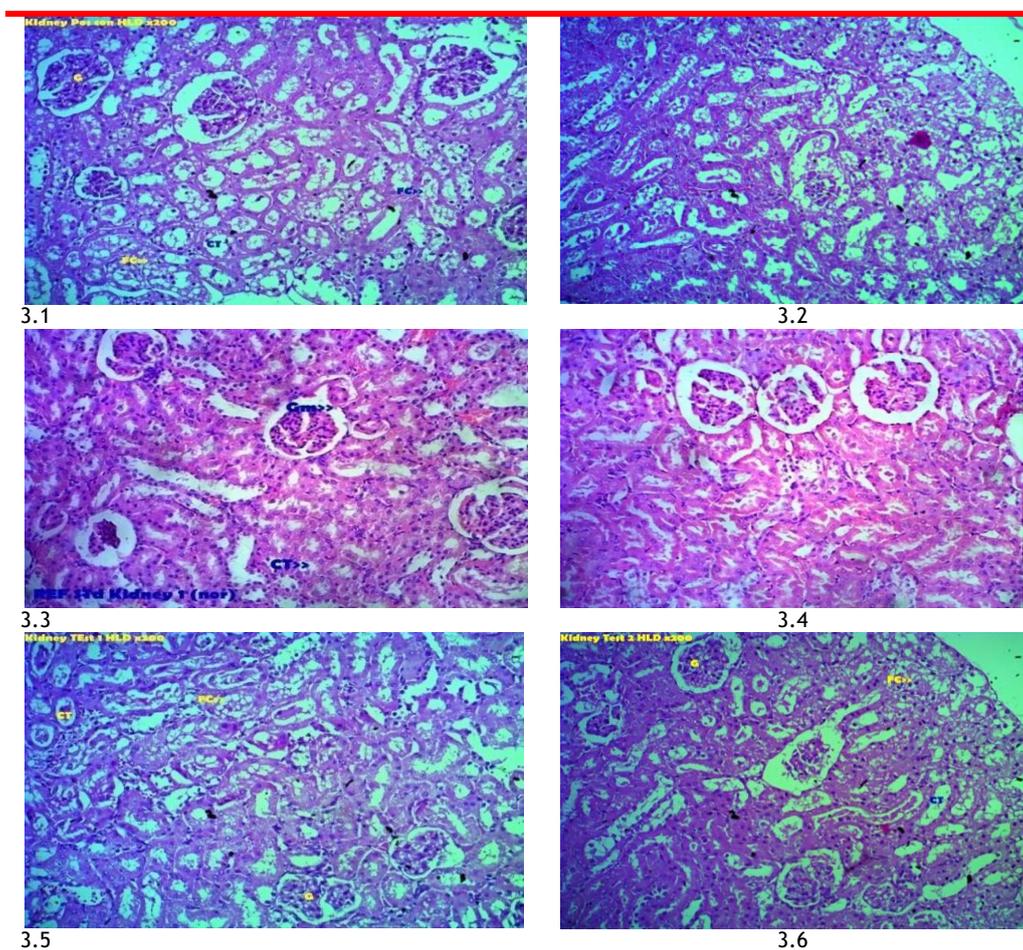
In the triglyceride metabolism diacylglycerol transferase (DAGT) and lipoprotein lipase plays an important role. Triglyceride synthesis is mainly regulated by diacylglycerol transferase (DAGT) in many tissues. Lipoprotein lipase (LPL) is the rate-limiting enzyme in the hydrolysis of triglyceride-rich lipoproteins.<sup>[17]</sup> In the present study triglycerides level is significantly reduced by Atorvastatin and test drug administered rats. Thus the test drug might have either inhibitory action on synthesis of TG by inhibiting DAGT or stimulating catalytic action of lipoprotein lipase.

There was marked increase in the level of serum biochemical parameters such as SGOT, SGPT and ALP in the high fat diet

administered rats as compared to normal control group. These elevated enzymes in hepatocytes usually released into circulation causing increase in the serum levels under hepatocellular injury or inflammation of the biliary tract cells. It has been reported that the hepatic damage induced in high-fat diet fed animal which could be rise in the level of SGOT and SGPT in liver and blood.<sup>[18]</sup> The test drug has failed to restore the elevated SGOT and SGPT to normal level but ALP level has been non- significantly decreased as compared to the high fat diet group.

Gradual gain in body weight indicates normal progressive health status of an organism. Decrease in the body weight is indicative of degenerative changes in the body or certain organs. In the present study animals administered with hyperlipidaemic diet have shown significant decrease in the percentage change body weight and relative weight of kidney as compared to normal control group. This indicates repeated administration of the hyperlipidaemic diet have caused degenerative changes in the body and kidney. However the test drug didn't show any significant changes in the relative body weight and organs weight such as liver, heart and kidney. The results were comparable with that of normal control group.

**Figure 3. Photomicrograph representative of kidney tissue**



Cholesterol control group (3.1 and 3.2), Reference standard treated with Atorvastatin (3.3 and 3.4), VISB represented in (3.5 and 3.6). CT: convoluted tubules, FC: fatty changes, CT: convoluted tubules, FC: fatty GC: glomerulus changes, G: glomerulus. Kidney exhibited mild to moderate fatty degenerative changes in cholesterol control group. In test group at 2 different doses the fatty degenerative changes were mild to moderate.

Histopathological report revealed that the hyperlipidaemic diet caused cellular changes in the vital organs like liver, heart and kidney. Liver cells have shown marked changes such as cell depletion, micro and macro changes in the hepatocytes and inflammatory changes in the lobules in the form of cell infiltration and edema. In some section ballooning of hepatocytes were also observed. But in Atorvastatin treated group above changes were

found to be markedly reduced. The test drug administration has shown only moderate reversal of hyperlipidaemic diet induced changes in the liver. Microscopic examination of heart section has shown normal cytoarchitecture in test drug treated groups. Kidney exhibited mild to moderate fatty degenerative changes in the high fat diet group. The fatty degenerative changes were moderately reversed by test drug administered at higher dose level.

#### 4. Conclusion

On the basis of above observation it can be concluded that co-administration of *Vateria indica* Linn. Seed butter has significant anti-hyperlipidemic action along with protective action on the vital organ systems. Hence the finding creates a scope of utilizing the traditional available seed oil as a food substance for internal administration along with other nutrient benefits. Further clinical study may give the accurate pattern of its metabolism and the result.

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**CONTRIBUTORS** Dr Rajmohan contributed to study design, literature study and data acquisition. Dr Suma and Dr Faisal contributed to the conceptualization of the topic, data analysis and manuscript editing. Ravi contributed to animal experiments, data analysis and manuscript review. Dr Ravishankar contributed to the intellectual content of study design and manuscript review.

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